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Marine fish cultivation: pioneering studies on the culture of the larvae of the plaice (*Pleuronectes platessa* L.) and the sole (*Solea solea* L.)

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Abstract

Early attempts to rear plaice (*Pleuronectes platessa* L.) in closed circulation conditions at the Lowestoft Fisheries Laboratory were followed in 1960 by similar work in open circulation at Port Erin. Technical adjustments from year to year improved larval survivals and experimental consistency to the point where a mass-production exercise could be undertaken with a reasonable chance of success. Between 1964 and 1967 both plaice and sole (*Solea solea* L.) were reared in quantities sufficient to support development work at experimental fish-farm sites in Scotland. Some examples of empirical sea-fish husbandry are given; research and development priorities are discussed.

ntroduction

narine hatchery project, motivated by the notion that local overfishing could be liorated by the release of fish eggs and larvae into coastal waters, began in America he 1870s and spread to other (mainly European) countries. It failed to establish the ctical value of artificial recruitment and declined after 80 years' operation. The his- cal details were reviewed by Shelbourne (1964). Few advances of note on the rearing narine fish larvae were made during this period, with the exception of a Norwegian ervation (Rollefsen, 1939) that the nauplius of *Artemia salina* L. (the brine shrimp) a suitable food for larval plaice.

A series of empirical rearing experiments on the plaice began in closed circulation at Lowestoft Fisheries Laboratory in 1957, using eggs caught at sea in townets. These estigations have been described fully by Shelbourne *et al.* (1963) and Riley and acker (1963). During five successive spawning seasons, laboratory output rose from o 2 807 metamorphosed larvae, at a maximum survivor density of 336 young fish · m^2 of tank bottom; survivals seldom exceeded 5 per cent of the original number of -spawned eggs.

In 1960, a second series of plaice-rearing experiments in open circulation, using pond- wned eggs, started at the Marine Biological Station, Port Erin, Isle of Man. As a ult of technical improvements over three successive seasons, the maximum survival es from the early egg stage, through and beyond larval metamorphosis, increased m 10.3 per cent to 65.7 per cent, at a maximum survivor density of 3 650 young fish r m^2 of tank bottom. After 1963, experimental objectives were influenced by the spec- problems of an emerging mass-production programme, designed to transform our pirical plaice-rearing technique into a commercial technology. During the course of s exercise, sole larvae were found to be suitable for cultivation in hatchery conditions. The main objective of the experimental series was to study the influence of important vironmental variables on the survival of flatfish larvae in captivity. Detailed accounts some individual Port Erin experiments have already been published (Shelbourne 63a, b), but shortened versions are included below for the sake of continuity.

The comparative ease with which larval mass-production problems were overcome Port Erin encouraged the White Fish Authority (UK) to undertake further develop- nt work at two experimental fish-farm sites in Scotland. There remains, however, a eat deal of supporting research yet to be completed before the commercial production farmed sea fish can become a reality. The nature and tactics of associated research and velopment programmes are discussed in the final section of this paper.

Plaice-Rearing experiments

All the experiments described below were conducted in replicate glass tanks (Figure 1) 60×30×30 cm in size, with an operational depth of 20 cm hatchery sea water. Each tank was equipped with a black polythene film jacket to cut out side lighting, a controllable drip-feed seawater inlet pipe and a screened overflow duct. Flow rates were standardized at 4 litres per hour per tank filtered seawater from a constant-level reservoir. Emergent larvae were fed, to slight excess, on the nauplii of *Artemia salina* (the brine shrimp) up to the completion of population metamorphosis, when the diet was often varied with cultured *Artemia* adults or white worms (*Enchytraeus albidus*).

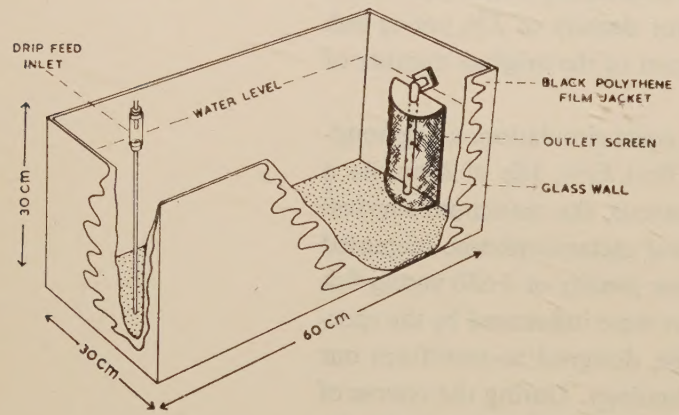


Fig. 1. A standard plaice egg incubator and larval-rearing tank.

Except where stated, a particular experiment would be stocked with pond-spawned flatfish eggs taken from a single egg collection on a particular day, so as to rule out genetic variation from tank to tank. At the end of each experiment, survivors were killed and preserved in 5 per cent formaldehyde in sea water for later analysis.

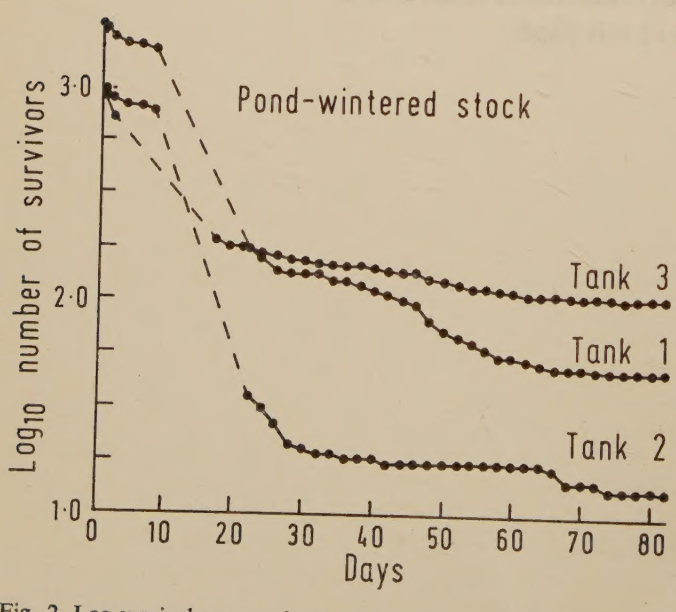
Experiment A, 1960: Preliminary work in open circulati conditions

Aim To test the Lowestoft closed-circulation techniqu rearing plaice in open circulation at Port Erin, using from pond-wintered or sea-wintered captive spawners.

Procedure Six standard tanks were stocked with egg p lations of variable origin and development status (see T 1). Tanks were illuminated by natural daylight reflected f hatchery walls and ceilings, occasionally augmented by o head tungsten lamps on dull days. Water temperature uncontrolled, increasing from 9°C during incubation 17°C at metamorphosis; the salinity remained steady 33–34‰; the pH (8.0–8.2) was within the tolerance ra quoted by Bishai (1960).

TABLE 1 *Survival data from a plaice-rearing experiment open circulation; Experiment A, 1960*

	Tank number					
	1	2	3	4	5	6
Egg stock	2 000	1 000	1 000	1 000	1 000	1 00
Hatched eggs	1 596	820	881	878	919	94
Established feeders	181	38	177	126	240	24
Metamorphosed survivors	47	13	101	70	93	10
Survivor density (numbers per m²)	261	72	561	389	517	572
Survivor length (nearest mm below)						
Minimum	12	12	10	11	11	10
Maximum	36	39	29	35	31	37
Mean	22	22	19	21	19	19
Standard deviation±	6.49	9.18	4.21	5.27	4.35	4.8



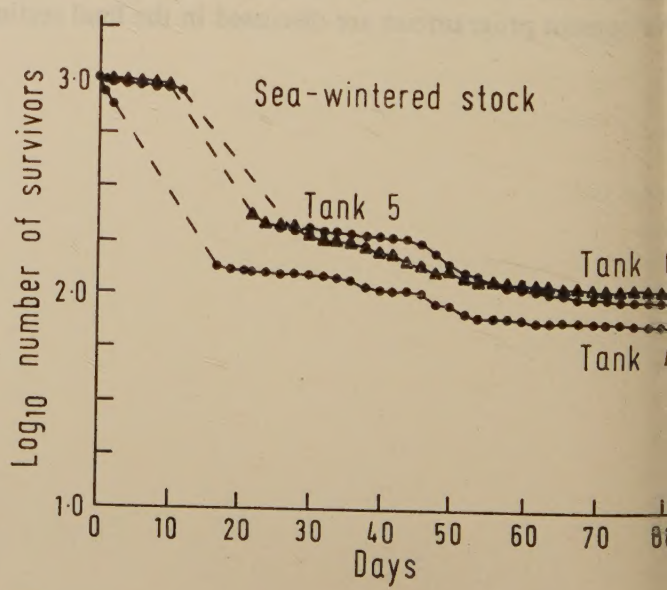
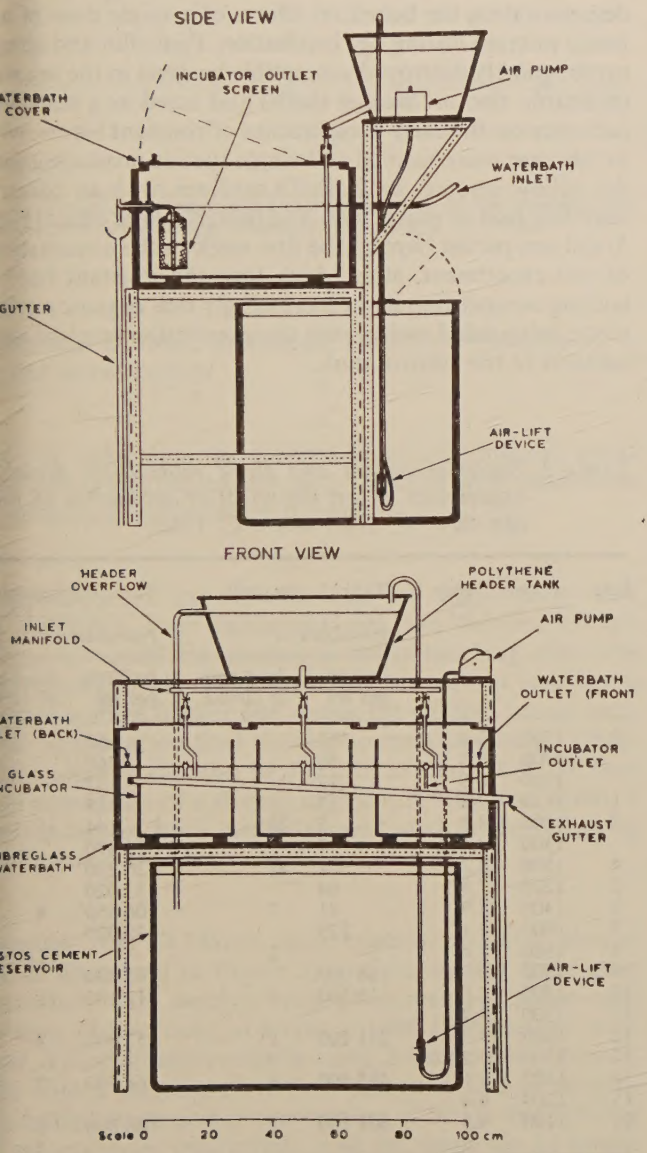


Fig. 2. Log-survival curves of tank-reared plaice stocks. Experiment A: 1960.

lts The basic numerical information of Table 1 is re-ced by the log-survival curves in Figure 2. The mean ival rate of 6.1 per cent compared favourably with a 2.7 ent survival in the same season at Lowestoft, using sea-ned eggs in closed circulation, survivor densities being l at 137 fish per m². Tanks 3–6 had mean survivals oaching 10 per cent, in contrast to low survivals in s 1 and 2, which were due to faulty tank design. The al stock in tank 3, from eggs spawned by pond-wintered ts, suffered no design hazard; its survival curve was lar to those from tanks 4, 5 and 6 (sea-wintered stock). r experiments by Bowers (1966) produced positive evi-e of differences in egg and larval viability relatable to adult environment before spawning.

eriment B, 1961: Salinity and larval survival

To study the influence of salinity fluctuations on the ival of pelagic plaice larvae.



3. Triple-tank plaice-rearing unit. Experiment B: 1961

Procedure Three units of three standard irrigated tanks (Figure 3) were provided with an equivalent degree of water temperature control; each tank was stocked with 1 000 plaice eggs in the gastrula stage of development, from a single pond collection. Tanks were illuminated from a fluorescent source. Twelve days after hatching, when the surviving larvae were strong enough to oppose any tendency to sink in diluted sea water, the salinities in units 1 and 3 were decreased from 34‰ to 32‰ over a period of 3 days; the salinity in control tank 2 remained steady at the hatchery norm (see Figure 4). Tank salinities were returned to normal, after which test stocks were subjected to a second period of salinity flux during the late stages of pelagic life and subsequent metamorphosis.

Results Numerical survival data for each tank are presented in Table 2, and mean log-survival curves for each unit of three standard tanks in Figure 4, together with salinity and temperature records. Significant increases in mortality among test stock coincided with the first salinity change; the effect is seen in Figure 4 as a prolongation of mortality for units 1 and 3 between hatching and the establishment of a strong feeding stock. The second salinity change did not influence test stock survival. A repeat experiment in 1962 yielded a negative result, suggesting that the 1961 effect may have been due to unknown background causes, or simply a reflection of the poor physiological condition of the early larvae in that year.

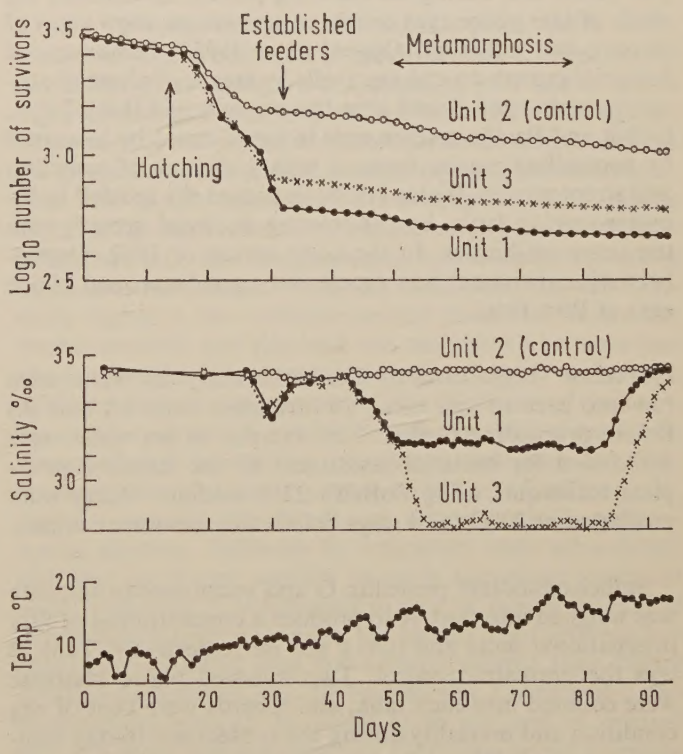


Fig. 4. Log-survival curves and salinity data for each unit of three plaice-rearing tanks, with mean water temperatures. Experiment B: 1961.

All tank populations showed an improvement in performance over former years, particularly the control series of unit 2 which yielded a mean survival of 33.3 per cent of the original eggs, at a mean survivor density of 1 850 young fish per m². Technical refinements included more effective illumination during the period of early larval feeding after exhaustion of yolk reserves. Later work by Blaxter (1968) confirmed a light intensity of 500 mc at the water surface to be optimal during this phase of development.

TABLE 2 *Survival data from a salinity experiment in open circulation; Experiment B, 1961*

	Unit 1			Unit 2 (control)			Unit 3		
	Rearing tank								
	1	2	3	4	5	6	7	8	9
Egg stock	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000
Hatched eggs	870	873	843	924	915	912	863	871	886
Established feeders	165	267	185	531	545	427	358	249	207
Metamorphosed survivors	126	203	138	333	356	311	287	174	139
Survivor density (metamorphosed fish per m^2)	700	1 127	766	1 850	1 977	1 727	1 594	966	772
Survivor length (mm)									
Minimum	10	11	13	10	10	10	10	11	12
Maximum	35	31	42	38	35	35	35	35	37
Mean	21.1	18.6	20.3	18.4	17.6	17.7	16.6	19.4	20.1
Standard deviation±	5.65	4.48	5.02	4.90	4.89	4.95	3.82	4.44	4.59
Light intensity (mc)	140	285	484	500	344	290	500	527	377

Experiment C, 1962: Antibiotics and egg survival

Aim To determine the effect of antibiotic treatment on plaice egg survival.

The eggs of the plaice often become opaque and adhere one to another during development in hatchery tanks. When the symptoms are severe, heavy mortalities can be expected just before and during the hatching period. In contrast, the shells of late plaice eggs caught at sea seldom show signs of opacity and adhesion. Oppenheimer (1955) demonstrated bacterial growth on cod egg shells by means of phase photomicrographs, and found that the percentage hatch of cod, turbot and Pacific sardine eggs in tanks could be increased by controlling marine bacteria with a mixture of penicillin and streptomycin. Walne (1958) improved the spatfall in his oyster-rearing tanks by suppressing bacterial growth with the same antibiotics. In the early spring of 1962, Oppenheimer's experiment was repeated on pond-spawned plaice eggs at Port Erin.

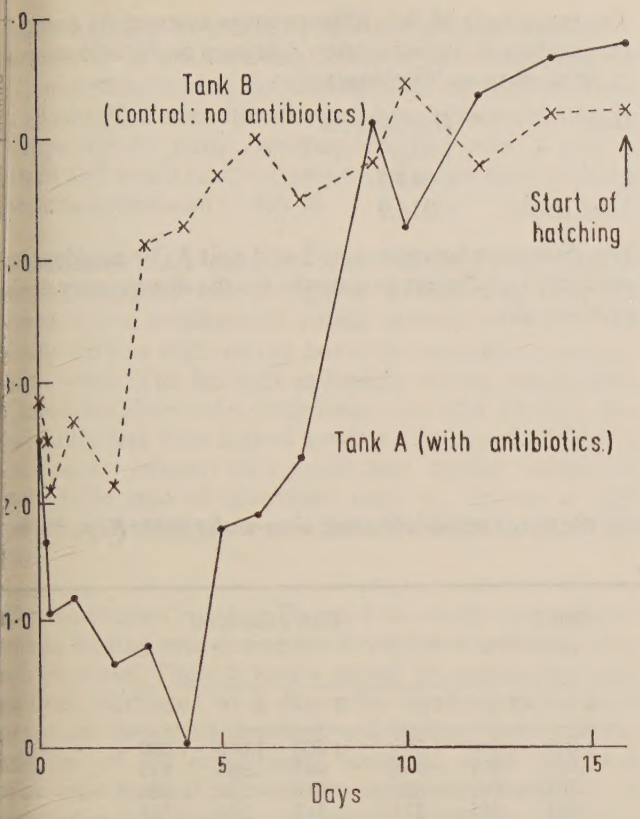
Procedure Eight litres of filtered hatchery sea water were run into each of two clean 10 litre glass tanks (A and B.) Before proceeding further, 1 ml samples of sea water were withdrawn for bacterial assessment by the standard pour-plate technique, using ZoBell's 2216 medium. Plates were incubated at 22°C for 4 days before colonies were counted.

Sufficient sodium penicillin G and streptomycin sulphate was weighed into tank A to produce a concentration of fifty international units and 0.05 g per ml respectively. Tank B was the untreated control. Two hundred plaice blastulae were counted into each tank, and records were kept of egg condition and mortality during the subsequent 16-day incubation period. Water samples were analysed for bacterial populations at regular intervals.

Results Temperature data, bacterial counts and stock mortalities are presented in Table 3. By day 4 the sea water in the untreated control tank B had, as expected, a definite odour connected with the proliferation of aerobes, and the plaice egg shells were turning opaque with a film of sessile bacteria. In contrast, the sea water in treated tank A was odourless and the egg shells were clear. Total egg losses in both tanks were high, demonstrating poor egg quality at the outset, underlined by the heavy mortality just after transfer and before gastrulation was complete (days 0–4). Subsequent losses were higher in the control tank B (36.9 per cent of maining stock) than in the test tank A (12.1 per ce demonstrating the beneficial effects of a single dose of a biotic mixture during egg incubation. Penicillin and strep mycin quickly destroyed susceptible bacteria in the sea wa (probably also on the egg shells) and acted as a restrain influence on the early proliferation of resistant forms, wh in this case was observed as a single species producing sm dry yellow colonies on ZoBell's medium, with an odour sembling that of musty hay. The bacteriostatic effect (Fig 5) did not persist beyond the first week in the circumstan of this experiment, after which time the resistant bacte lacking competition in an increasingly rich organic envir ment, rebounded well above the population level of mi bacteria in the control tank.

TABLE 3 *Bacterial counts and stock mortalities during experiment to test the effect of antibiotics on pla egg survival; Experiment C, 1962*

Day	Time	Temp. (°C)	Tank A (treated)		Tank B (control)	
			Numbers of		Numbers of	
			bacteria per ml	dead eggs or larvae	bacteria per ml	dead e or larv
0	1500	6.4	367		662	
	1700	6.5	50		360	
	1900	6.6	13		127	
1	1130	7.8	17		489	
2	1500	8.3	5	26	144	31
	1300	8.4	7		13 870	
4	1500	8.2	1	42	20 230	50
5	1200	7.8	64		52 020	
6	1300	8.9	81	7	106 350	4
7	1800	8.1	229		30 920	
8	1900	8.8		2		11
9	1800	8.7	138 000		65 800	
10	1500	8.6	18 100	3	312 100	16
11	1300	8.7				
12	1600	9.3	231 200	1	57 600	7
13	1300	9.2				
14	1400	9.1	485 500	1	168 700	2
15	1200	9.4				
16	1100	9.5	601 100	2	146 800	4



. 5. The effect of penicillin and streptomycin on the proliferation bacteria in a plaice egg incubator. Experiment C: 1962.

periment D, 1962: Antibiotics, ultra-violet irradiation; g and larval survival

m To determine the survival value of antibiotic treatent during egg incubation, and irrigation with ultra-violet adiated sea water during subsequent larval life.

Oppenheimer's experiments, and the confirmatory test st described, were concerned with the survival of fish mbryos in antibiotic solution as far as hatching only. The wly-emerging plaice-rearing technique made it possible to vance a stage further and assess the effects of early antiotic treatment on final survival to metamorphosis. Ultraolet radiation can also be effective in controlling the bacrial population of a storage reservoir (Shelbourne 1964); a mbination of both methods was tested in the following periment.

ocedure On 23 March, three experimental units similar that illustrated in Figure 3, each containing three glass cubators, were stocked with pond-spawned plaice eggs ss than 24 hours old, at a rate of 1 000 blastulae per incutor. The incubators in units 1 and 2 were treated immediely with a mixture of sodium penicillin G and streptoycin sulphate to give concentrations of 50 i.u. and 0.05 mg r ml sea water respectively, and left static for 24 hours. nit 3 was the untreated control. Unit 1 was then irrigated th hatchery sea water previously sterilized by ultra-violet ght, whilst unit 2 was irrigated with normal hatchery water. ght days after the start, unit 1 received a second dose of tibiotics. The treatment during egg incubation can be mmarized as follows:

Unit 1 2 doses of antibiotic mixture; irrigated with ultra-violet treated sea water at a rate of 4 litres (10 per cent operational volume) per hour.

Unit 2 1 dose of antibiotic mixture; irrigated with normal hatchery sea water at above rate.

Unit 3 no antibiotics; irrigated with normal hatchery sea water at above rate.

Incubation temperatures were not strictly controlled, but buffered by a slow circulation of hatchery sea water around the incubators contained in the fibre-glass units. Despite this precaution, temperatures were above optimum, lying within the range 8.5–9.0°C at hatching, 11–12°C at first-feeding and 14–15°C at metamorphosis. Incubators were illuminated somewhat unevenly, by fluorescent lighting from 0900 to 2100 hours each day.

In 1962 an electronic device had been developed for counting *Artemia* nauplii (Mitson 1963). Measured rations were offered daily to tank stocks from the fifth day after hatching, in quantities sufficient to provide a small residual food population after 24 hours' feeding. Tanks were cleaned regularly; water samples were analysed for marine bacteria at frequent intervals. The experiment ended on 23 June (92 days) when all survivors were fully metamorphosed.

Results Survival data for each tank are presented in Table 4, and the fate of each unit population (sum of three tanks) can be followed in Figure 6. Treated units 1 and 2 held a slight advantage at hatching (97 per cent hatched as against 95 per cent in the untreated controls); hence plaice embryos are tolerant to penicillin and streptomycin at the concentrations used in this experiment. Some early larval mortalities occurred in unit 1, caused by air bubbles being carried into incubators with inflowing sea water. By the time non-feeding mortalities had ceased on day 40, mean survivals were generally higher in the antibiotic-treated tanks than in the untreated controls and this lead was maintained to metamorphosis. For the 3-week period between days 40 and 60 the relative survival rates, represented by the slopes of the semi-log curves in Figure 6, indicate that the benefits of antibiotic dosage during incubation are carried over into the free-swimming larval phase (compare units 1 and 2 with unit 3); it is also seen that double dosage with a penicillin-streptomycin mixture, followed by irrigation with ultra-violet-sterilized sea water, confers an early advantage (compare unit 1 with unit 2). Subsequent to day 50, during the period of population metamorphosis, the treated units continued to compare favourably with the controls despite their higher population densities, but the lead of unit 1 over unit 2 was considerably reduced. Survivals of 46 per cent of original eggs at a mean survivor length of 17–18 mm and at a density of 2 500 young fish per m^2 were unprecedented and constituted an encouraging advance towards a consistent plaice-rearing technique.

Plate cultures of 1 ml water samples taken during the course of the experiment once again indicated the presence of penicillin-streptomycin resistant bacteria giving small yellow colonies with the distinctive smell of musty hay. Although the ultra-violet system (unit 1) gave a 100 per cent kill of bacteria in the seawater inflow, contaminants were introduced with larval food and these proliferated in the rearing tanks to produce bacterial populations no less dense

than in the controls (Figure 7). The sterilization of inflowing seawater with ultra-violet radiation might provide a means of controlling hatchery bacteria, if used in association with other aseptic precautions. Its value as employed in the above experiment cannot be said to have been established by the final results.

The display of survivor length (Table 4) is of some interest; the mean size of metamorphosed fish from the antibiotic treated tanks was consistently higher than in the control tanks. The mean daily temperature of all units combined is given in Figure 6; in practice the control tanks ran at a slightly lower overall temperature than the treated units.

The magnitude of this difference was assessed by comp the product of day's duration × degrees centigrade, com ly referred to as 'day/degrees':

Unit	*Day/degrees*
1	1 051.9
2	1 043.9
3 (controls)	1 033.9

The difference between unit 2 and unit 3 (10 day/degre probably insufficient to account for the discrepancy in r survivor size.

TABLE 4 *Survival data for a plaice-rearing experiment incorporating the use of anti-biotics and ultra-violet light; Experimer 1962*

	Unit 1			Unit 2			Unit 3 (control)		
	Rearing tank								
	1	2	3	4	5	6	7	8	9
Egg stock	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000
Hatched eggs	979	984	963	981	979	966	943	964	955
Established feeders	799	668	824	782	691	618	630	588	666
Metamorphosed survivors	467	389	468	462	455	337	317	246	303
Survivor density (metamorphosed fish per m²)	2 594	2 161	2 600	2 566	2 527	1 872	1 761	1 366	1 683
Survivor length (mm)									
Minimum	10	12	10	11	11	11	10	10	10
Maximum	30	30	28	29	31	32	26	26	27
Mean	17.3	18.0	17.1	17.5	18.6	18.4	16.4	16.7	16.4
Standard deviation±	3.7	3.6	4.0	4.1	4.4	4.9	3.4	3.5	3.1
Light intensity at water surface (mc)	300	360	370	360	410	340	370	520	490

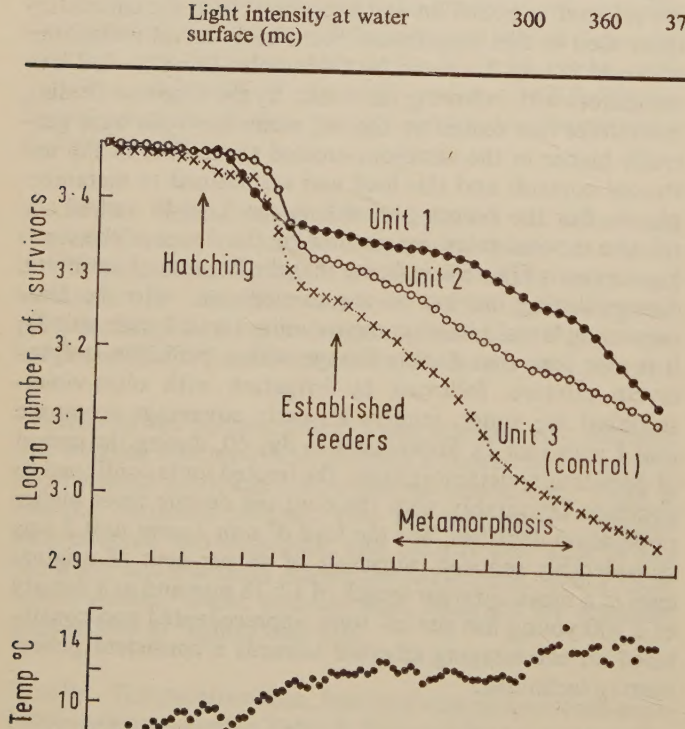


Fig. 6. Log-survival curves of tank-reared plaice stock and mean water temperatures. Experiment D: 1962.

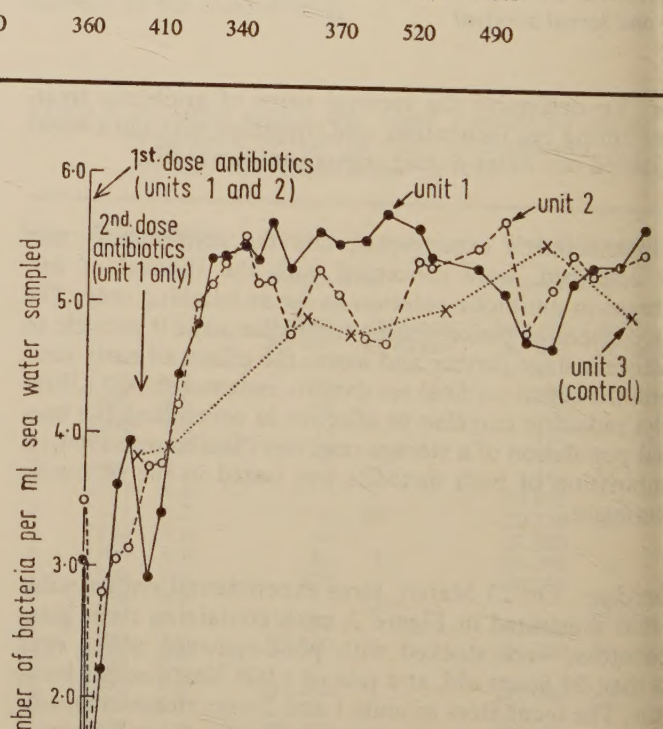


Fig. 7. Mean bacterial populations in the plaice-rearing units Experiment D: 1962.

gure 8 gives the mean daily ration of *Artemia* nauplii per . offered to the surviving stock of each experimental (comprising three tanks) during 2-day intervals between 19 and 92. The continuous and discontinuous lines give approximate daily consumption for units 1 and 2 ted) and unit 3 (control) respectively, and show marked rences subsequent to day 58.

ie control stock suddenly went 'off feed', for reasons h, at the time, were thought to be associated with the rrence of a troublesome ciliate infestation. Unit 2 was affected to a slight extent, but made a complete recovery in a week. This set-back in feeding activity among con- larvae explains why their mean size after 92 days was ificantly less than that of survivors from units 1 and 2. se tanks treated with antibiotics during incubation ved little sign of infestation later on, because a high dard of tank hygiene was easier to maintain from the et.

he continuous curve in Figure 8 shows the main fluctu- ns in feeding rate during the larval life of a plaice popu- on in tanks. There is first a period of accelerating con- ption, starting 4 to 5 days after hatching, when yolk rves are becoming depleted, and lasting 30 days in the ditions of the experiment. Secondly, there follows a ilar time interval between the onset and completion of amorphosis within the population, when the rate of in- ase in naupliar consumption falls off. Thirdly, after the jority of larvae have metamorphosed, feeding activity elerates to a new rate of increase in naupliar consumption alling that of period 1.

n open circulation conditions, some loss of naupliar food ough, or on, the outlet screen of a fish-rearing tank is vitable, even in conditions of slow flow. The mean feeding ve for units 1 and 2 is therefore probably biased on the h side; also, it relates to the temperature regime given in ure 6. Nevertheless, the plot has been used successfully organize the logistics of larval food supply in later mass- duction exercises.

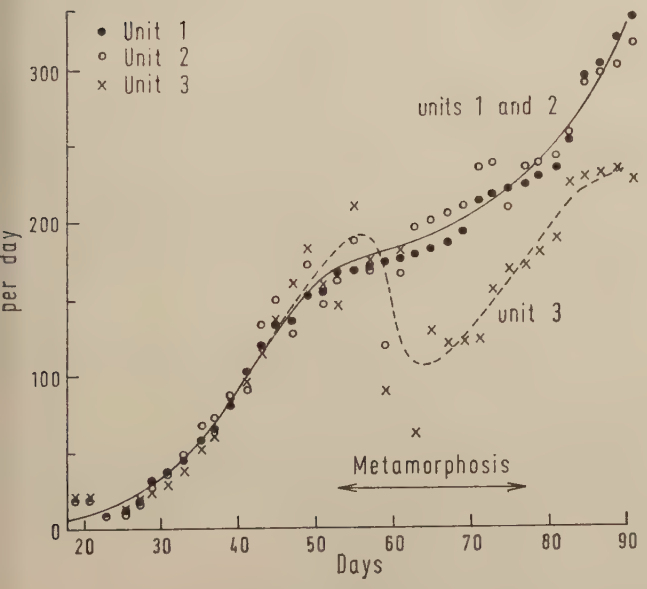
Other studies of naupliar consumption by plaice larvae at Port Erin in 1962 were made by Riley (1966). His data (Figure 9) give a deeply interrupted curve similar to that for unit 3 in Figure 8, which he interpreted as representing a cessation of feeding by stock, consequent on the structural strains of metamorphosis. This might be valid if all larvae in a tank population metamorphosed at the same instant. In fact, the individuals of a heterozygous plaice population undergo larval development at different rates, and any fall-off in individual feeding at structural metamorphosis would, for a large stock, appear as a flattening of the feeding curve rather than a trough. I regard Riley's data as representing the feeding activity of a temporarily infested or infected stock.

Experiment E, 1962: Antibiotics, temperature control and larval survival

Aim To determine the effect of antibiotic treatment during egg incubation on the subsequent survival of plaice larvae in temperature-controlled conditions.

The usefulness of penicillin-streptomycin treatment was also demonstrated in a second experiment, planned as part of a long-term study of temperature effects on plaice egg and larval survival.

Procedure Four freshwater baths, similar in design to that shown in Figure 3 (but lacking lower reservoir and upper header tank), were constructed. Each bath contained two partially-immersed standard glass incubation tanks, the middle tank of Figure 3 having been removed to accommodate a copper cooling unit linked to its own domestic refrigeration unit. A good circulation was maintained over the cooling coil by recycling the water in the bath through a small centrifugal pump, and the temperature was controlled at a level less than ambient, by means of a sensitive contact thermometer activating a control relay connected to the refrigerator motor.



. 8. The mean ration of *Artemia* nauplii per larva per day fed to ice stock in the rearing units of Experiment D: 1962.

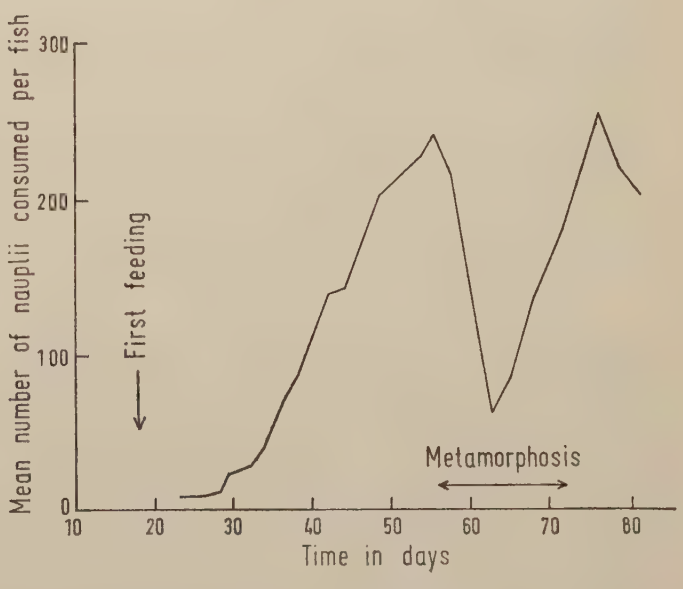


Fig. 9. The mean consumption of *Artemia* nauplii by plaice larvae in the control tanks of a feeding experiment, according to Riley (1966).

Each glass incubator (60×30×30 cm) could be irrigated with hatchery seawater pre-adjusted to the temperature of the freshwater bath. This was achieved by conducting the inflowing seawater through a 15 m polythene coil (6 mm bore) immersed in the water-bath, before entry into the incubator. Seawater overflowed through a standard right-angled outlet pipe, the horizontal limb of which was carried through the glass tank wall and the face of the water-bath, to discharge into a drain gutter. The apparatus was designed to minimize the risk of admixture between the seawater and the freshwater systems. Rearing tanks were illuminated and serviced in a similar manner to that of the previous experiment.

On 22 March, each of the eight incubators was stocked with 1 000 pond-spawned blastulae, selected from a single egg collection. Although tanks could be supplied with hatchery water overflowing to waste, only one tank of a pair (tank B) was irrigated during the period of egg incubation. The other (tank A) was treated with one dose of penicillin and strepromycin at the concentrations already stated, and then kept static until hatching began. All tanks were irrigated during the subsequent larval phase.

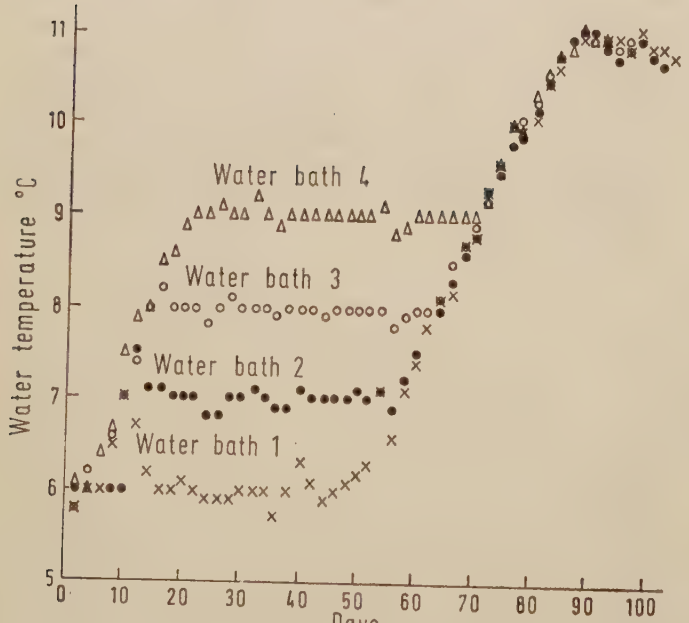


Fig. 10. Water bath temperatures for Experiment E: 1962.

The temperature regime for each water-bath is indicated in Figure 10. After 2 days' acclimatization at 6°C, the temperatures of three water-baths were slowly increased, at a mean rate of 1°C per week, to give 6, 7, 8 and 9°C in water-baths 1, 2, 3 and 4 respectively at the time larval feeding began. These temperatures were maintained through the critical early feeding phase, which was of longest duration at the lowest temperature (water-bath 1). Thereafter, the lower temperatures were slowly raised to 9°C, followed by an increase in all baths, at an equal rate, to a maximum of 11°C at metamorphosis.

Daily counted rations of *Artemia* nauplii were adjuste as to maintain a small residual food population in rearing tank after 24 hours of larval feeding. Bacteriolo analyses of sea water were not made, but a high standa tank hygiene was maintained. Tank survivors were k and preserved when no more than 3 per cent remained pe ic:

Water-bath	Tank	Date experiment ended	Duration (days)	Proportion of survivors metamorphosed
1	A	4 July	104	97
	B		104	98
2	A	2 July	102	97
	B		102	98
3	A	28 June	98	99
	B		98	99
4	A	22 June	92	98
	B		92	99

Results Stock survivals are displayed in Table 5 and Fig 11. At all temperatures a higher percentage hatch occur in tanks treated with antibiotics, although differences tween treated and control tanks were not as marked as in t experiments of Oppenheimer (1955). Slight differences survival between treated and untreated egg stocks w magnified after hatching and during larval feeding, at temperatures, indicating that the benefits of antibiotics d ing incubation continue to be felt long after hatching complete. Embryos are not necessarily killed by shell co taminants, but are undoubtedly weakened; in each case, t treated stock produced the more competent feeders. Arg ments based on laboratory experience, supporting the id of a corresponding 'critical' phase in the sea, must inevitab be weakened by the results of this experiment. The artific hazards of the tank environment clearly influence the amp tude of non-feeding mortalities. A critical period will st occur in the sea, however, if suitable planktonic food scarce during the early larval stages.

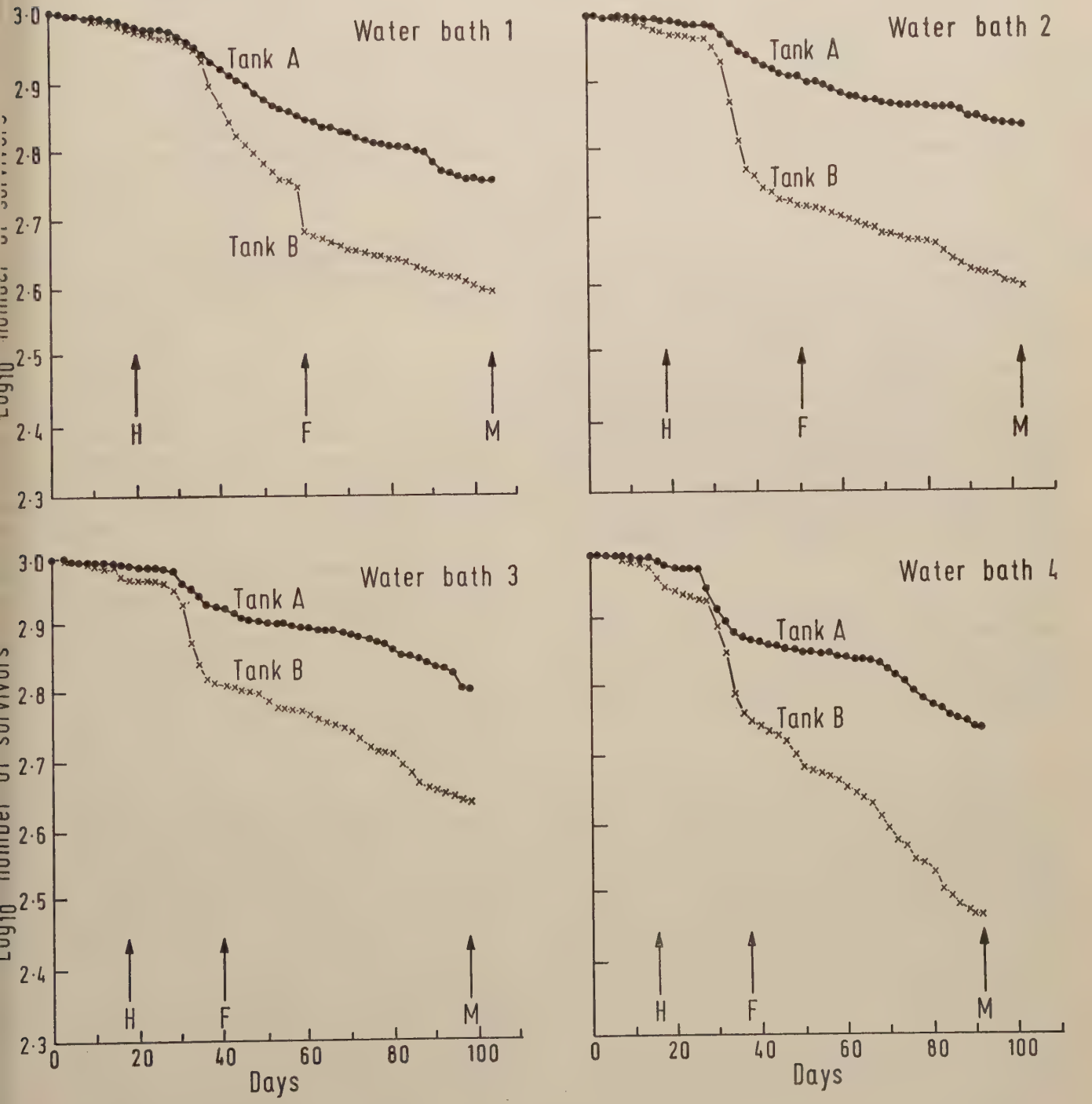
Final survivals per tank are best compared when expe mental stocks are in equal states of development. The da degree indices given in Table 5 are a measure of develo ment status, and show that survivals in water-baths 2 and are directly comparable. Using these indices as a standar stocks in water-bath 1 were slightly less advanced, and water-bath 3 slightly more advanced, at the end of the e periment.

The sum totals of both metamorphosed and pelagic su vivors for each water-bath were:

Water-bath			
1	2	3	4
968	1 068	1 065	853

n allowing for slight discrepancies in development status clear that the regimes in water-baths 2 and 3 conferred e advantage on stocks. Relating these figures to the rse of survival in each tank (Table 5 and Figure 11), it ld appear that the optimum temperature for plaice egg bation, using Irish Sea stock, lies within the range °C and that a shift to 8°C during early feeding is bene-l. Thereafter, a steady temperature increase at a rate of more than 0.5°C per week up to the completion of meta-rphosis is likely to minimize mortalities among feeding l metamorphosing stock.

The survival in water-bath 2 (treated tank) was very high indeed—65.7 per cent of original egg stock passed safely through metamorphosis, giving a survivor density of 3 650 young fish per m^2 of tank bottom at a mean length of 14.9 mm. At these densities (about 3 per square inch), post-metamorphosis competition is fierce, and continuing mortalities are usual under such conditions of high population pressure. This contrasts with the flatness of the post-metamorphosis survival curve at lower survival rates and densities in earlier experiments.



g. 11. Log-survival curves of plaice stock with and without antibiotic treatment during incubation, and in variable temperature conditions. xperiment E: 1962.

TABLE 5 *Survival data from a temperature-controlled plaice-rearing experiment using anti-biotics; Experiment E, 1962*

	Water-bath							
	1		2		3		4	
	Rearing tank							
	A	B	A	B	A	B	A	B
Egg stock	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000
Hatched eggs	966	942	976	937	975	932	968	911
Established feeders	706	481	788	516	823	643	738	556
Survivors								
(a) metamorphosed	561	387	657	387	632	426	548	297
(b) pelagic	14	6	19	5	2	5	7	1
Duration × temperature °C (day/degrees)	813.0	816.1	828.6	831.3	839.4	840.7	827.1	829.2
Survivor density (metamorphosed fish per m²)	3 116	2 150	3 650	2 150	3 511	2 366	3 044	1 650
Survivor length (mm)								
Minimum	10	10	10	10	10	10	10	11
Maximum	22	24	22	25	27	25	23	21
Mean	14.1	14.2	14.9	14.5	15.0	14 1	15.0	13.9
Standard deviation±	2.0	2.2	2.4	2.5	2.6	2.2	2.4	2.0
Light intensity (mc)	494	538	398	494	484	505	527	494

A final survival of 65 per cent is far removed from the former notion that plaice could not be reared ashore (Petersen 1894), and it narrows the gap between expectations in marine and freshwater fish hatcheries. There is no reliable evidence on the survival rate of plaice to metamorphosis in the sea. Gross (1950b) produced a figure of 0.1 to 0.2 per cent survival of newly-hatched fry liberated into a specially enriched area of a Scottish sea-loch, but, as he pointed out, his calculations did not take into account the probable immigration of young plaice from outside the loch. It is safe to assume that the rearing technique practised at Port Erin in 1962 yielded survivals of young plaice at least several hundred times greater than normal survival rates in the open sea.

Experiment F, 1963: Population density, size composition and larval survival

Aim To study the effect of (a) population density and (b) culling procedures, on the performance of plaice larvae in hatchery tanks.

Most experiments described so far were stocked at an arbitrary rate of no more than 1 000 plaice eggs per 60 × 30 × 30 cm glass incubator (5 555 eggs per m² water surface). By 1963, tank design and management procedures had improved to the point where population density experiments could be undertaken with a reasonable chance of producing clear results. These were combined with a second exercise to determine the effect on overall survival of separating a mixed population of developing larvae of equal age into fast- and slow-growing fractions.

Procedure Six standard glass incubators contained in two fibre-glass water-baths (Figure 3), through which hatchery seawater was circulated for cooling purposes, were stocked with plaice blastulae sorted from a single collection of pond-spawned eggs at the following rates:

Water-bath	Tank number	Number of eggs
1	1	1 000
	2	1 000
	3	2 000
2	4	2 000
	5	3 000
	6	3 000

Sufficient quantities of sodium penicillin G and streptomycin sulphate were added to each tank to produce a concentration of 50 i.u and 0.05 mg per ml respectively. Eggs were incubated in static conditions for 14 days at the prevailing temperature, until 90 per cent were hatched; tanks were then irrigated with filtered hatchery seawater at a rate of 4 litres per hour. Dead eggs and larvae were removed on alternate days, and a good standard of hygiene was maintained throughout the experiment. Four days after hatching *Artemia* nauplii were offered as food, the tanks being illuminated from 0900 to 2100 hours each day by overhead fluorescent lamps.

The larvae in tanks 1, 2, 3 and 5 were allowed to develop through and beyond metamorphosis undisturbed. When approximately half the larval population in tank 4 had entered the metamorphosis phase (day 58) and were taking to the bottom, the remaining pelagic larvae (the slow developers) were carefully removed into another standard glass tank, designated 4A. Tanks 4 and 4A were each fed half the daily ration offered to tank 3. Similarly with tank 6, two thirds of the stock comprising pelagic larvae were transferred to tank 6A on day 50, and this population was again divided on day 66 by removing the symmetrical pelagic forms into a third glass tank designated 6B. The daily ration of *Artemia* nauplii fed to tanks 6, 6A and 6B at any stage of the experiment were, in total, equal to the tank 5 ration.

The experiment lasted 92 days, when all surviving stock were killed and preserved for further analysis.

TABLE 6 *Stock transfer sequence and survival data for a population density experiment on plaice eggs and larvae; Experiment F, 1963*

Day		Rearing tank								
		1	2	3	4	4A	5	6	6A	6B
0	Egg stock	1 000	1 000	2 000	2 000		3 000	3 000		
18	Hatched eggs	946	941	1 881	1 896		2 828	2 809		
38	Established feeders	455	519	1 066	1 179		1 660	1 770		
50		435	493	992	1 120		1 578	1 686		
								510	1 176	
58	Surviving larvae at times of transfer	407	455	923	1 061		1 489	492	1 161	
					516	545				
66		374	385	832	486	542	1 351	484	1 135	
									560	575
92	Survivors									
	(a) metamorphosed	310	351	776	439	523	1 092	466	544	557
	(b) pelagic	4	2	6	2	6	10	0	1	9
	% survival	31.4	35.3	39.1	48.5		36.7	52.5		
	Duration × temperature °C (day/degrees)	1 009	1 010	1 011	1 009	1 001	1 009	1 014	1 002	998
	Survivor density (metamorphosed fish per m²)	1 772	1 950	4 311	2 438	2 905	6 066	2 588	3 022	3 094
	Survivor length (mm)									
	Minimum	10	10	10	11	10	10	10	10	10
	Maximum	28	26	27	22	20	28	23	20	20
	Mean	15.7	14.9	14.7	14.3	14.1	15.1	14.7	14.1	13.5
	Standard deviation±	3.2	2.6	2.8	1.9	1.8	3.4	2.0	1.7	1.8
	Light intensity (mc)	376	473	441	398	473	419	365	376	408

Results (a) Population density effects on survival The sequence of stock transfers and survival data are given in Table 6. Despite differences in tank population density there were no significant differences in egg mortality at the completion of hatching on day 18. By the time tank 6 was ready for culling on day 50, survival was best in the more densely populated tanks:

Tank number	Stocking rate per tank	Mean % survival on day 50
1 and 2	1 000	46.4
3 and 4	2 000	49.6
5 and 6	3 000	54.4

Final survivals for the undisturbed stock (tanks 1, 2, 3 and 5), with 99 per cent of all larvae metamorphosed, lay between 30 and 40 per cent; tank 2 at the intermediate stocking density had a slight advantage. The mean temperature regime (Figure 12) was not optimal, being on the high side during incubation, but the day/degree indices given in Table 5 indicate that tank stocks were of comparable developmental status at the end of the experiment.

The solid symbols (plus the open circles) in Figure 12 represent the log survivals for undisturbed stock at the three levels of population density. All curves show the usual characteristics; firstly, a steady slight egg loss during incubation; secondly, a period of increased mortality (day 24 to 40) up to the establishment of a strong feeding stock; thirdly, a further period of increased larval loss between day 56 and 66, coinciding with the onset of metamorphosis. All deaths during this latter period were of runted stage 4 larvae.

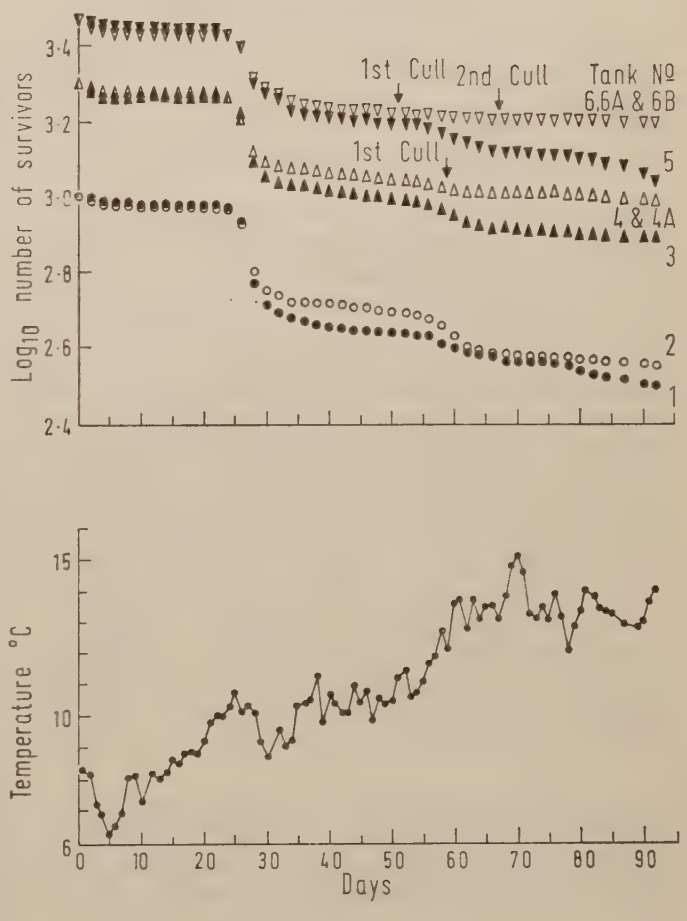


Fig. 12. Log-survival curves and mean daily water temperatures for Experiment F: 1963.

Up to day 80, high population density did not unduly influence the proportional death rate, but over the remaining 12 days of the experiment a disproportionate mortality occurred in tank 5, which contained the densest stock. In the conditions of this experiment, the maximum permissible egg stocking rate calculated to avoid population density effects in the later stages of larval life lies between 2 000 and 3 000 eggs per standard incubator.

(b) Effects of stock separation on survival The progeny of unselected plaice spawners from wild sources show characteristically wide variations in development rate during tank life. At metamorphosis the fast developers take to the bottom, and it is a relatively simple matter to remove the late-developing and still-pelagic larvae by beaker, siphon or pipette. By means of the first separation on day 50 and a second on day 66, the survivors in tank 6 were divided into three populations with different mean development rates (tank 6 > 6A > 6B). The mixed larval population in tank 4 was similarly re-distributed into fast and slow developers (4 > 4A) by the single separation of day 58. No unusual mortality, directly attributable to culling procedures, occurred.

The beneficial effects on survival of this separation technique can be ascertained from the data displayed in Table 6. Comparing the performance of undisturbed and separated stocks at the higher stocking density levels between the time of initial separation and the end of the experiment gives the following results:

Stock	Tank number	Number of		Percentage mortality during period
		larvae before separation	survivors on day 92	
Undisturbed	3	923	782	15.2
Separated	4+4A	1 061	970	8.5
Undisturbed	5	1 489	1 102	25.9
Separated	6+6A+6B	1 686	1 577	6.4

The improved survival of separated stock (open triangles) as against undisturbed stock (closed triangles) can be seen in Figure 12. It could be argued that the act of separation involved a change towards decreased population density and this alone might be the reason for reduced mortalities. The validity of such an argument can be tested by comparing the survival curves for tanks 1 and 2 (closed and open circles) with those for the separated stock. During the period between stock separation and the end of the experiment, population densities in the separated stock tanks were at a somewhat higher level than in tanks 1 and 2, containing undisturbed larvae. Even so, the slopes of the survival curves after day 50 are less steep for separated than for unseparated stock. One can conclude from this evidence that the mortality rate among feeding plaice larvae in a hatchery tank is influenced not only by the density of that population, but also by its size (or developmental) structure. Presumably, hierarchical patterns become established early in the life of a captive plaice population.

Experiment G, 1966: Irrigation, aeration and larval survival

Aim To compare the relative merits of irrigation and aeration on the survival of plaice larvae in hatchery tanks.

By 1964, arrangements had been made to transform the experimental technique for rearing plaice into a mass-production technology. As this work progressed, so the demand for hatchery seawater increased, reaching a critical level 1966. It then became necessary to rationalize the 'open circ lation' policy of former years.

The two main functions of water flow, in the context hatchery production, are (a) to complement gaseous diffi ion at the water surface by active transport of O_2 into tank, and (b) to restrict the rate of metabolite accumulati arising from the respiratory and feeding activities of liv stock, food organisms and bacteria in a tank. For maximu effect a fast flow is required; in practice, however, the irrig tion rate had to be balanced against the limitations of ta design.

The alternative method of O_2 supply by direct aeratio was known, from early experience, to cause heavy morta ties among newly-hatched larvae if employed carelessly. Le information was available, from either personal experien or documented sources, about the tolerance of plaice larv to their own metabolites or to those produced by the foo or other organisms present in the tanks.

Procedure Four pairs of standard 60 × 30 × 30 cm glass i cubators, each stocked with 1 000 blastulae (24 hours old) staggered intervals of time, were provided with variab conditions of water flow and aeration. The egg stock f each tank of a pair came from either an outdoor or an indo pond, as follows:

Tank number	Date stocked	Egg source	Treatment
17 18	14 March	Outdoor pond Indoor pond	Standard flow (4 litres/hou sea water after hatching: r aeration
19 20	2 March	Outdoor pond Indoor pond	Static throughout: no aeratio
21 22	5 March	Outdoor pond Indoor pond	Standard flow seawater aft hatching: aeration throughou
23 24	8 March	Outdoor pond Indoor pond	Static throughout: aeratio throughout

The aerators in tanks 21–24 each consisted of a diffuse stone attached to a 3 mm rigid plastic air tube, contained i a coarsely-perforated polythene cylinder (25 mm bore) su rounded by a close-fitting tube-like bag of black boltin silk. This fine-mesh bag was closed at its lower end near th tank bottom, but open above the surface of the water. Th polythene cylinder and bag were a simple device to prever contact between air bubbles and fish larvae. In those tank provided with continuous irrigation after hatching, the ir flowing sea water was conducted through a 6 mm bore blac polythene inlet tube to discharge near the tank bottom. A tanks were static during egg incubation in sea water contain ing a single standard dose of antibiotics; very gentle aeratio continued throughout this period in those tanks fitted wit protected diffusers, though when survivors were stron enough (day 70) the protective screens were removed to giv better aeration.

The experiment was carried out in a hatchery productio space already committed to a slowly-rising temperatur schedule. Because of the staggered stocking procedure, a element of variation was introduced into the temperatur regimes of particular pairs of tanks (Figure 13). Overhea fluorescent lamps provided adequate illumination for

riod of 12 hours each day (0900 to 2100 hours). For tech-:al reasons, the tank cleaning routine and removal of dead is restricted to an average frequency of once every 4 days ring the 82 days' duration of the experiment. Larval food *rtemia* nauplii) was offered daily, in abundance, from 4 ys after the start of hatching in each tank.

esults: Survival Numerical data are given in Table 7 and e survival curve for each tank is plotted semi-logarithmic-ly in Figure 13. It is obvious immediately that the initial action to handling of eggs from the indoor pond was more arked than that of outdoor eggs. The indoor stock of aice spawners was housed in a $3.65 \times 3.65 \times 1.22$ m fibre-ass tank continuously irrigated by a copious flow of atchery sea water, and had been fed a regular diet of opped 'queens' (*Chlamys opercularis*) since their transfer 7 months before the experiment started. The act of spawn-g was not inhibited by the indoor environment, but liber-ted eggs were, without exception, soft-shelled – in contrast the eggs from outdoor spawners. The relative shell tough-ess of representative egg samples, measured by the mean ressure (in grammes) required to burst the shell, showed a onsistent variation with origin:

Tank number	Sample of 50 eggs representing	
	indoor origin	outdoor origin
17		199
18	33	
19		173
20	26	
21		177
22	25	
23		122
24	26	

It is appropriate to state, at this point, that in 1967, after a further 12-month period of acclimatization to indoor conditions, the same spawners produced eggs with exceptionally tough shells.

Despite the formation of imperfect shells during 1966, the eggs from indoor spawners in tanks 18 and 24 yielded survivals to metamorphosis at the 50 per cent level. The outdoor eggs (ignoring the special case of tank 19) gave a better survival grouping after 82 days, but cannot be considered as being intrinsically more viable than the indoor egg stock.

The second noticeable feature of Figure 13 is the steepness of the mortality slopes for tanks 19 and 20 (static: no air) after day 60, at two different levels of population density. Up to that day the oxygen tension in both tanks was evidently sufficient to meet the needs of larval survival; by day 82 the dissolved oxygen concentration had fallen to less than 0.3 ml/litre as measured by the Winkler titration (< 5.5 per cent saturation at the prevailing temperature and salinity).

Oxygen depletion also probably occurred in tanks 23 and 24 between days 65 and 70, producing an acceleration in larval mortality. The subsequent stabilization of both survival curves followed an improvement in tank aeration by the removal of aerator screens. It is remarkable that the larvae in these static (though aerated) tanks were able to withstand their own metabolite concentrations as well as they did; indeed, it was static tank 23 which produced the highest final survival for outdoor eggs.

Two extra variables were introduced into the experiment by staggering the egg stocking times: (a) a genetic variation from tank to tank, since eggs were probably spawned by different parents, even when collected from the same pond,

TABLE 7 *Survival data for plaice stocks from an irrigation/aeration study; Experiment G, 1966*

Day		Treatment							
		Static +air		Flow +air		Static: no air		Flow: no air	
		Egg origin							
		I.*	O.**	I.	O.	I.	O.	I.	O.
		Rearing tank							
		24	23	22	21	20	19	18	17
0	Egg stock	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000
18	Hatched eggs	756	924	886	914	508	969	904	894
45	Established feeders	574	567	375	454	391	680	611	512
82	Survivors								
	(a) metamorphosed	499	486	350	419	94	313	561	419
	(b) pelagic	2	3	0	8	1	13	1	0
	% mortality (day 45–82)	12.7	13.7	6.7	5.9	75.7	52.0	8.0	18.2
	Duration × temperature °C (day/degrees)	959	944	920	919	893	895	993	993
	Survivor density (metamorphosed fish per m^2)	2 772	2 700	1 944	2 327	522	1 738	3 116	2 327
	Survivor length (mm)								
	Minimum	10	10	10	10	10	10	10	10
	Maximum	24	28	23	25	26	23	31	29
	Mean	17.1	16.4	16.5	15.6	16.5	14.1	16.2	18.0
	Standard deviation±	2.7	3.2	2.8	3.1	3.5	2.4	3.8	4.1
	Light intensity (mc)	355	441	411	436	436	410	409	452

*I. (Indoor)=eggs from indoor spawning pond.
**O. (Outdoor)=eggs from outdoor spawning pond.

and (b) the temperature variation already alluded to, and reflected in the day/degree indices in Table 7. The influence of (a) and (b) on any assessment of flow/aeration effects will be great if final survivals are used as a basis for comparison. A more realistic assessment can be reached by comparing proportional mortalities between day 45, when all survivors were feeding strongly, and day 82, the end of the experiment (Table 7). In order of rank, the beneficial effect of flow+air > flow only > static+air > static only, although the difference between flow only and static+air is negligible.

From the standpoint of large-scale hatchery producti these results suggested that the continuous irrigation rearing tanks (an expensive measure) might be replaced b policy of gentle tank aeration coupled with intermittent fl without seriously affecting survival rates. In 1967, ei plaice production tanks (capacity 200 litres) were ea stocked with 4 000 blastulae from the same egg collecti and treated with antibiotics. After hatching, tanks 1 and (controls) were irrigated continuously at a rate of 16 lit per hour, whilst the other three pairs were subjected to sin

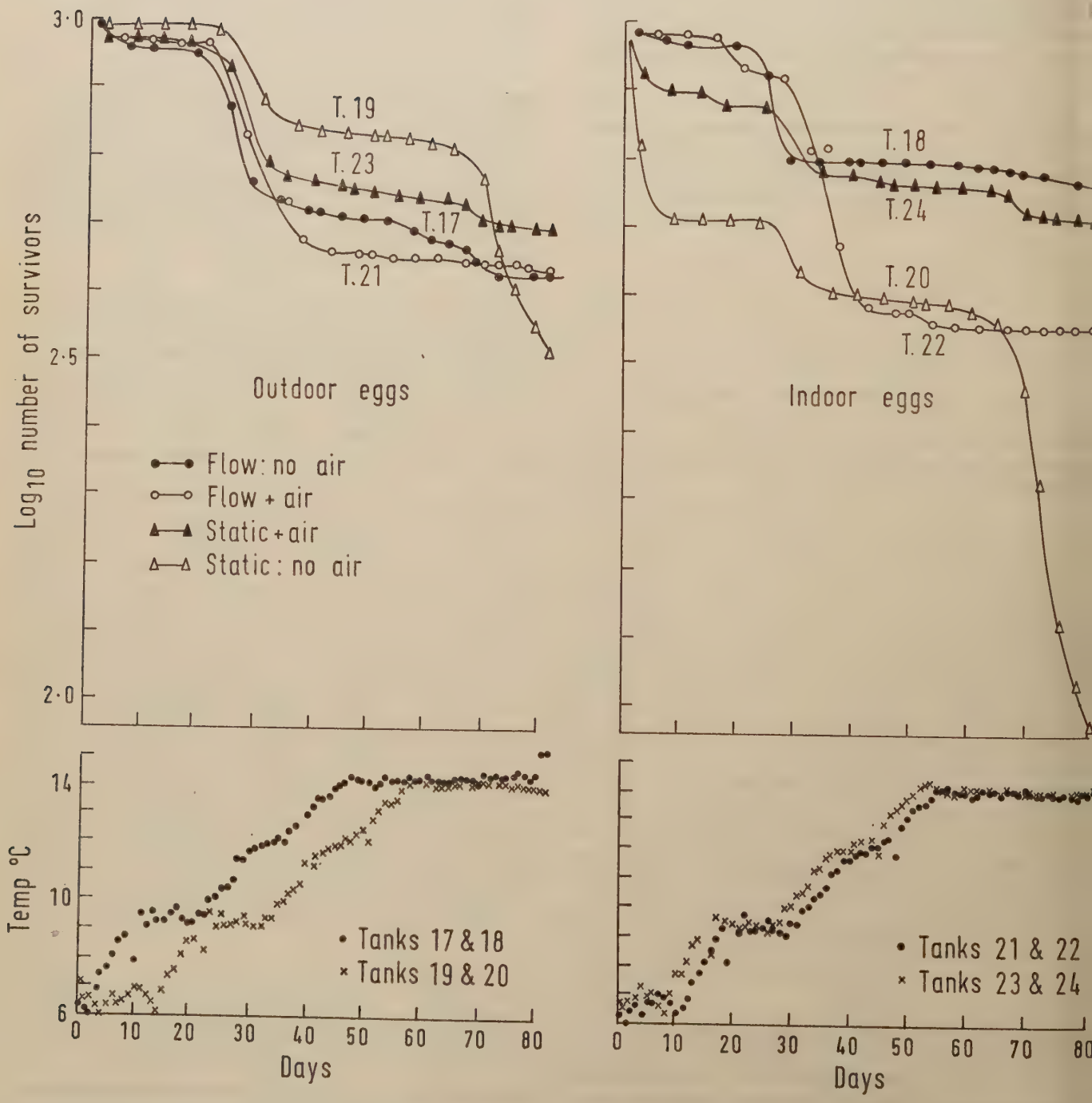


Fig. 13. Log-survival curves for all tanks, and mean water temperatures for tank pairs. Experiment C: 1966.

Plate 1. The marine hatchery at Port Erin, Isle of Man, with outdoor spawning ponds.

Plate 2. A array of plaice-rearing tanks at the Port Erin hatchery.

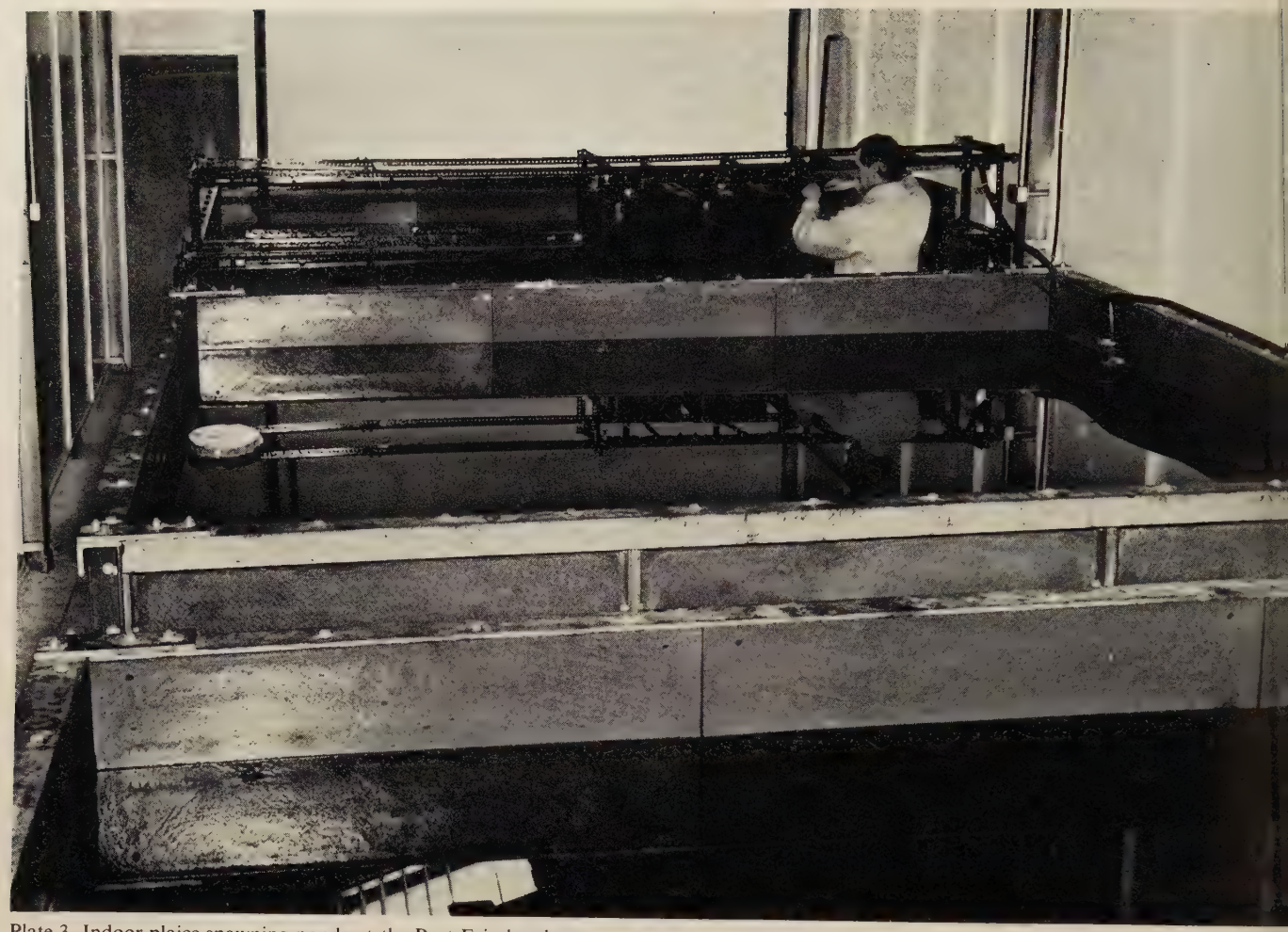


Plate 3. Indoor plaice spawning-ponds at the Port Erin hatchery.

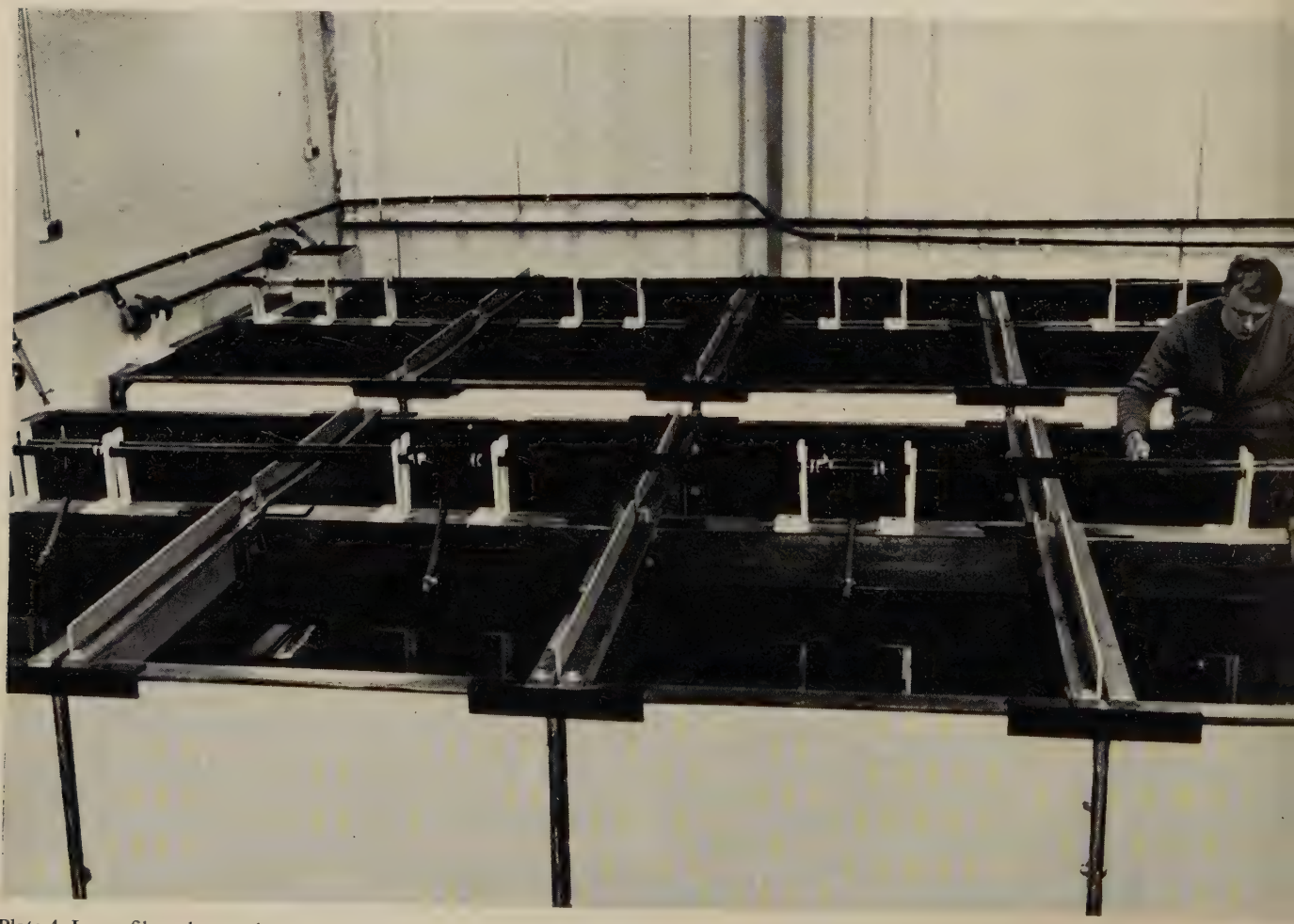


Plate 4. Large fibre-glass tanks used for the large-scale production of metamorphosed sole.

Plate 5. Submerged netting tanks supported by rafts, used for farming the yellow-tail *(Seriola quinqueradiata)* at Hiroshima Japan.

Plate 6. A shore-line yellow-tail enclosure near Takamatsu, Japan.

Plate 7. The netting barrier at the entrance to Matsumigaura Bay, Lake Hamana, Japan.

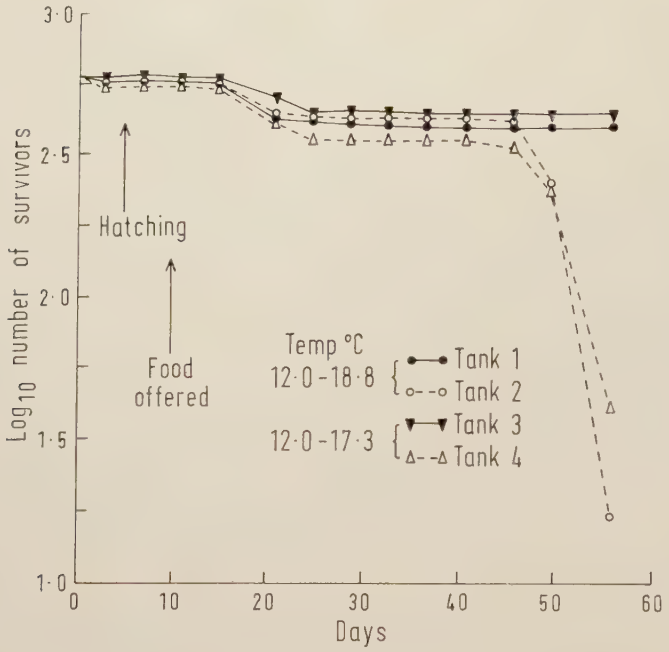


Fig. 15. The comparative survival of sole larvae fed on *Artemia* nauplii of either San Francisco or Utah origin.

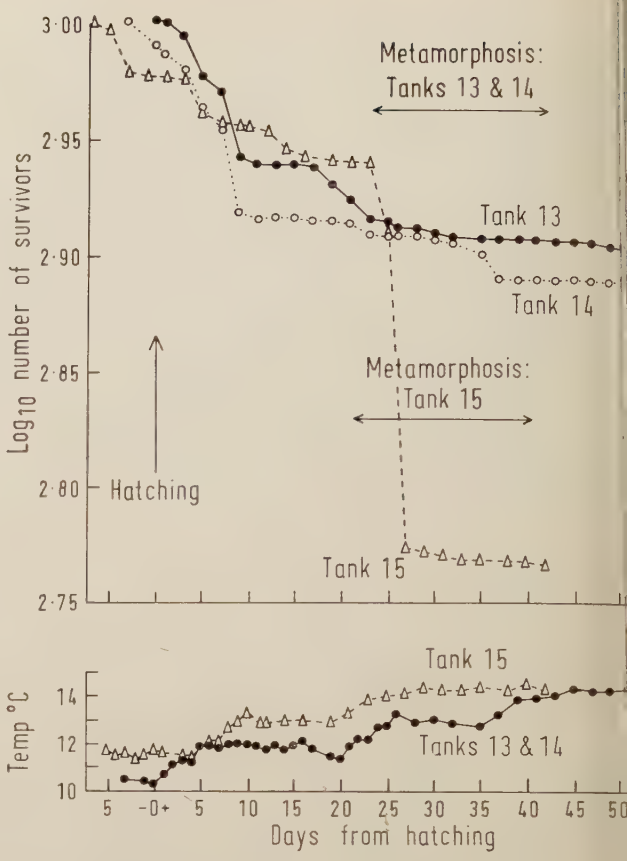
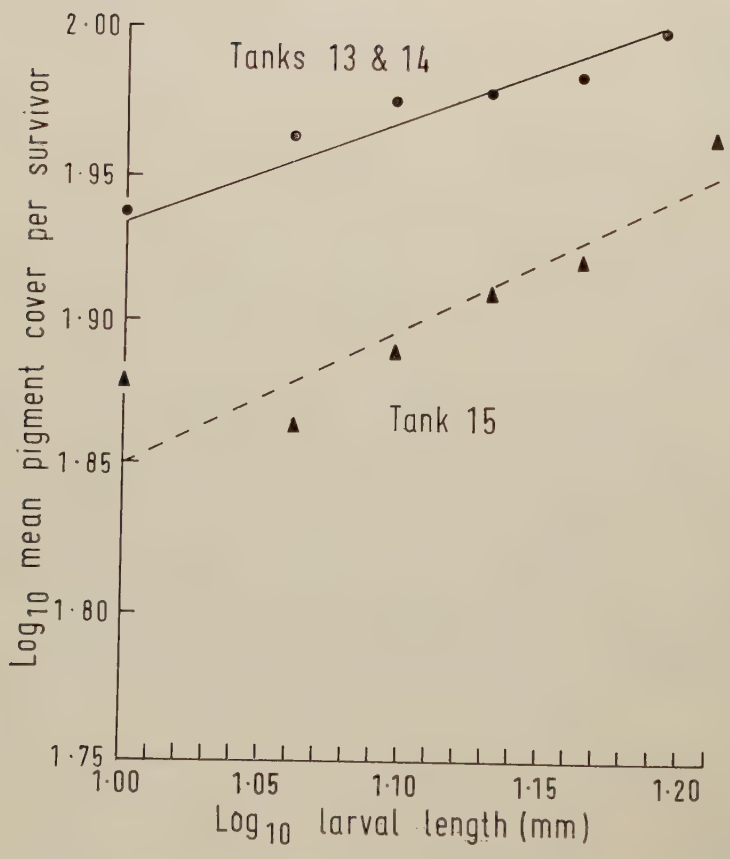


Fig. 16. Log-survival curves for sole eggs and larvae. Experiment H: 1965



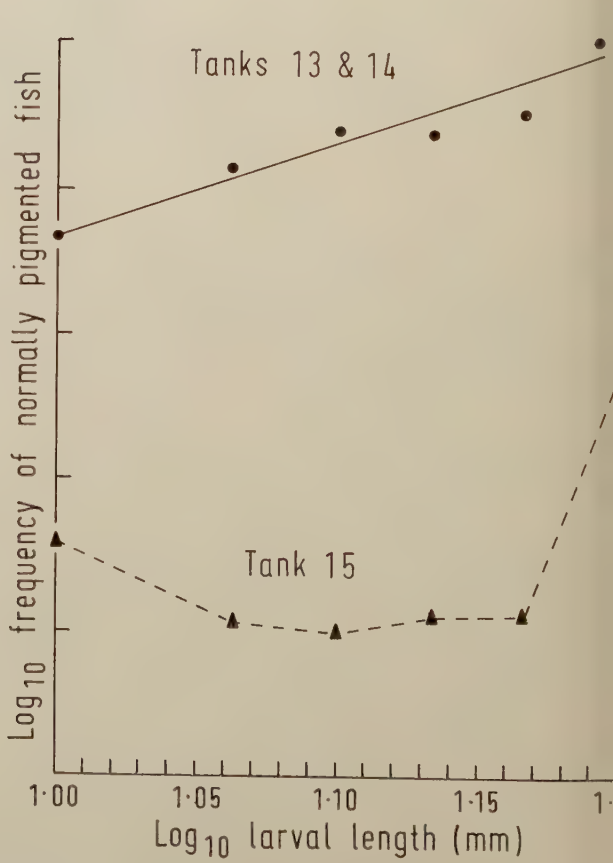


Fig. 17. The logarithmic relationship between dorsal pigment cover and survivor length, for the sole. Experiment H: 1965.

our periods of irrigation at the same rate of flow, but at ly differing intervals of time. During irrigation, each tank received a volume of hatchery sea water equal to wn operational capacity. All tanks were aerated gently feeding started; temperature, feeding and cleaning nes were equalized. The following result was obtained 91 days:

uction tank	Irrigation schedule	% survival to metamorphosis
1 2 (controls)	Continuous standard flow	24.4
1 4	A single irrigation daily	29.3
1 6	A single irrigation each week	24.6
1 8	A single irrigation each month	34.9

mass-production purposes, a plaice hatchery technique rporating continuous aeration after 'first-feeding' and equent water renewal seems adequate from the stand-nt of larval survival to metamorphosis. It is cheaper than olicy of continuous irrigation, avoids loss of naupliar d through outlet screens, and should have the advantage simplifying precautions against the introduction of hogens into rearing tanks.

cussion

e early developmental stages of flatfish have certain draw-ks as experimental material. Larvae are equipped to lead olitary, non-shoaling life in the vastness of the open sea; hatchery environment, on the other hand, involves con-ement and overcrowding, which must make great de-nds on the adaptational resources of larvae. The genetic iability among progeny from a wild stock determines perimental design to some extent; tank populations rather in individual fish are more usually under test, and there is ow limit to the number of tank populations that can be en adequate surveillance, even by an experienced cultur-. In the absence of controlled spawning techniques, experi-ntal material is only available during a short spawning son; if a technical breakdown occurs during the later ges of an investigation, it is usually too late to make a sh start that season. Theories derived from observations ring one season cannot usually be tested until the follow-g year. These difficulties explain the paucity of useful in-rmation on the laboratory requirements of marine fish vae, up to the time when sustained work began at westoft in 1957.

The first four experimental seasons were spent learning w to avoid technical mistakes. Tank design, water cir-itry and routine procedures were taken to a point where e chances of completing a seasonal programme without a ajor set-back were good. Annual survival rates remained w, seldom exceeding 10 per cent of original eggs, but by 61 sufficient general observations on the response of eggs d larvae to known hatchery conditions had been accumu-ted to suggest the quickest route to high survivals. Techni-l improvements were required in the following sectors:

tank illumination, particularly at the time of 'first-feeding', 4 to 5 days after hatching;

the temperature regime, particularly during the period of incubation;

egg condition (a) in relation to the bacterial environment during incubation;

egg condition (b) at the time of spawning, by better acclimatization and welfare of captive spawners.

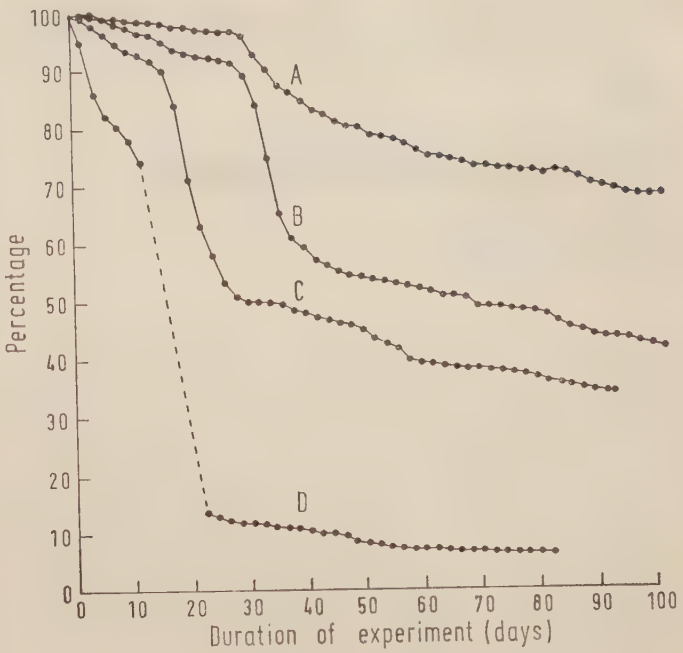


Fig. 14. The improvement in plaice survival (expressed as a percentage of original egg stock) during the period 1960-1962 at Port Erin.

During the 1961 and 1962 seasons at Port Erin, improvements were made in aspects (1), (2) and (3) of the egg and larval environment. The effect of these changes on survival rate is recapitulated in Figure 14. Higher survivals were accompanied by greater experimental consistency, best exemplified in Figure 11, and were repeated the following year in the population density/stock separation experiment. Work by Bowers (1966) has elucidated aspect (4), the importance of spawning stock acclimatization to the viability of their eggs and larvae in hatchery conditions. In 1962, at Port Erin, his acclimatized plaice stock produced eggs giving a mean survival to metamorphosis of 20 per cent, compared with a 4 per cent survival from unacclimatized spawners.

Populations of metamorphosed plaice reared in the hatchery are characterized by peculiarities of size distribution, incidence of bitten fins and dorsal pigmentation. The evidence has been reported briefly by Shelbourne (1963a, b; 1964), Shelbourne *et al* (1963), and Riley and Thacker (1963), and in more detail by Shelbourne (1974). For present purposes it is sufficient to state that pigment abnormalities are of frequent occurrence among tank-reared plaice. The smaller, more slowly-developing larvae of a tank population show this abnormality to a greater degree than the larger, faster-growing ones; its incidence increases with increased population density above a threshold level. Also, the stability of larval pigment seems to be related to the physiological condition of spawning stock at the time of egg liberation.

A total of 25 000 young plaice were produced in 1962 from environmental experiments of one sort or another; a survivor density at metamorphosis exceeding 6 000 young fish per m^2 of tank bottom occurred in tank 5, Experiment F, during 1963. Not only, it seemed, was a mass-production technology well within the bounds of possibility, but the scale of facilities necessary for a yield of, say, 1 million young fish per annum did not appear to be unmanageable.

The Mass-Production of Metamorphosed Flatfish

There are several different ways in which the output of a marine hatchery could be utilized to augment the national supply of sea fish; these will be discussed in a later section. All require a consistent technology for the annual production of young flatfish on a very large scale indeed, unless wild fish are sufficiently abundant in the sea to meet the local demand for seed stock by future commercial producers. The short-term reliance on wild fish for stocking purposes, unaccompanied by hatchery activity, would naturally prejudice the longer-term prospects of product improvement by selective breeding.

Hatchery design

During 1963 the encouraging outcome of our hatchery research programme began to attract public funds for a development project aimed at transforming the experimental technique for rearing flatfish into a mass-production technology. A new hatchery financed jointly by the White Fish Authority and the Ministry of Agriculture, Fisheries and Food was constructed on a site adjacent to the Marine Biological Station at Port Erin. It is an insulated, prefabricated structure (Plate 1), $30 \times 10 \times 4$ m to the eaves, covering a space divided into four main sections by light partitions:

(a) the hatchery proper ($15 \times 10 \times 4$ m);

(b) the spawning pond area ($15 \times 5 \times 4$ m);

(c) a larval food production unit ($5 \times 5 \times 4$ m);

(d) a general laboratory ($10 \times 5 \times 4$ m).

(a) Half the available space in the hatchery proper was occupied by a total of 128 black, polythene fish production tanks ($120 \times 60 \times 30$ cm deep), arranged in rows and tiers on a light, galvanized-steel frame-work (Plate 2). Each tank could be gravity-fed with filtered hatchery seawater, and was fitted with a screened overflow/outlet system draining into discharge channels in the floor. When tanks are arranged one above another in this fashion, each requires an independent source of illumination—provided by 40 watt fluorescent tubes. The heat output from 128 lighting units was dissipated by six heat exchangers sited in the roof peak and powered by two 3 h.p. compressor/condensers. The hatchery air temperature was thermostatically controlled at frequently-adjusted levels, sufficient to maintain tank water temperatures within the optimum range throughout the rearing season, even in conditions of maximum seawater flow (5–10 per cent tank volume per hour) and high ambient air temperature.

(b) The spawning area comprised three ponds ($3.65 \times 3.65 \times 1.22$ m deep) constructed from sectional fibre-glass panels (Plate 3). Each pond was illuminated by dim natural lighting through translucent roof windows, and was irrigated by a steady flow of unfiltered hatchery seawater. A pond of this size can hold 30 to 40 medium-size plaice or sole adults (30–40 cm length).

The fecundity of the plaice is more closely related weight and the cube of its length than to its age (Sim 1951). A female plaice, 35 cm long, from the Irish Sea liberate 100 000 eggs (Bagenal 1960); taking the Irish stock as an example, then 20 medium-sized females theoretically produce all the eggs needed to stock a hat designed to rear 1 million metamorphosed fish at a 5 cent survival rate. In practice, it may be necessary to cu developing larvae to eliminate incompetent forms. Ev in the process, final survival diminishes to 20 per cent o original eggs, a mere 50 captive females could meet req ments. Dannevig (1910) stocked ripe female and male c the ratio 4:1. If the same sex proportions give 100 per fertilization in the case of the plaice, then no more tha fish need be maintained from year to year for a target duction of 1 million metamorphosed larvae.

(c) An effective production technique demands adeq solutions to the logistic problems of larval food supply. *Artemia* nauplii production unit at Port Erin was house an insulated space controlled at a temperature of 23–2 It consisted of a fully automated double incubator sys capable of delivering 200 million nauplii per day, an out sufficient to feed 1 million metamorphosing plaice or s The unit was controlled from a monitored console wh relayed timed impulses in an orderly sequence to the vari components of the system (magnetic valves, aerators, w agitators, floodlamps, etc.) during the 48 hours' incubat period. *Artemia* eggs are obtained from American sour though unexploited reserves occur elsewhere in the wo wherever summer salt lakes are to be found. The pres (1971) cost of this imported food is less than 1p per metam phosed fish.

Technical procedures, 1964–67

The details of hatchery management varied from year year, according to the outcome of associated experimen studies. As a general rule, daily collections of newly-spawn plaice eggs from both indoor and outdoor spawning por were transferred to holding tanks containing antibiot treated seawater for a period of 48 hours after capture, allow eggs damaged during collection to sink out. Hatche production tanks were then stocked with weighed quantit of buoyant eggs and left in a static state (with antibiotics) 6°C until hatching. In 1964–65, tanks were continuou irrigated with hatchery seawater during the larval phase; 1966–67, influenced by the results of Experiment G, t continuous flow technique was superseded by a regime intermittent flow and continuous gentle aeration. Egg stoc ing densities were high during the first two years of produ tion, but the combined depressive effect on survivor pigme of poor spawner acclimatization and larval overcrowdin forced a reduction to 4 000 eggs per tank in 1966 and 196

Rations of *Artemia* nauplii were fed daily to each ta stock in quantities sufficient to maintain a slight surpl after 24 hours' feeding. Forecasting larval food requiremen for successive stages of development proved a relative simple task, with the assistance of Figure 8. Tanks we cleaned once per week, and the final stock of metamo phosed fish per tank was assessed by sampling the popul tion density on twenty evenly-distributed and equal areas tank bottom viewed through a special eyepiece.

al output

ress at Port Erin towards transforming an experimental ng technique for plaice into a mass-production tech- gy for metamorphosed flatfish can be summarized as ws:

Year	Target Production		Estimated production	
	Plaice	Sole	Plaice	Sole
1964	250 000	—	161 000	—
1965	500 000	—	375 000	15 000
1966	50 000	50 000	3 000	5 000
1967	40 000	20 000	55 000	58 000

ual survival rates for plaice larvae remained steady at 30 per cent of original eggs; the incidence of normally- nented fish rose from 10 to 80 per cent of all survivors ng the 4-year period, with increasing acclimatization of dent spawning stock and decreasing target densities.

ncouraging results in 1964 and 1965 influenced a decision he White Fish Authority to provide experimental facili- for cultivation work at Ardtoe, west Argyllshire (plaice), Hunterston nuclear generating station, Ayrshire (sole). special requirement of field sites for 20–30 mm 0-group rather than 15 mm newly-metamorphosed stock began determine target production levels after 1965.

Production targets were not met in 1966, due to wholly oreseen circumstances. We normally use *Artemia* eggs m the San Francisco region; these were unobtainable in ly 1966 because of business changes in California, and ernative supplies were purchased from commercial pro- cers in Utah. Heavy larval mortalities began to occur ee weeks after introducing Utah nauplii into the rearing ks. Later experiments, which were restricted to the sole ause of the non-availability of plaice eggs, proved the bility of *Artemia* from the Great Salt Lakes to support od survival and growth of flatfish larvae (Figure 15). New *temia* supplies from San Francisco arrived too late to save re than a few thousand weak survivors and their recovery s not complete. The shortcomings of the Utah food have en investigated by Bookhout and Costlow (1970), who ieve its toxicity to developing crab larvae to be a conse- ence of high DDT content.

For economic reasons, the current trend in marine tchery development at Port Erin and associated farm sites away from replicate tanks of small-size, operating in con- lled conditions, towards larger fish-rearing units func- ning in relaxed environmental circumstances. Survival es may fall, but eggs are expendable. A first account of val sole performance under the new system is given in the xt section.

The Artificial Propagation of the Sole

The sole is of particular interest to marine fish culturists, since it commands a consistently high market price sustained, no doubt, by relative scarcity, fine texture and flavour, and good keeping qualities. Unlike plaice, which can be categorized as an Arcto-boreal species with a characteristically explosive spawning period (Qasim 1955), the sole prefers warmer Mediterranean-boreal waters, and, though equally fecund, has a protracted spawning, often extending into the early summer. The male sole is seldom found in a 'milty' condition during the reproductory season. Sperm maturation and liberation is probably limited to the act of egg fertilization in nature, and for this reason artificial fertilizations are difficult to secure. Indeed, Cunningham (1890) was obliged to use a dissected and incised whole testis in his efforts to fertilize a small sample of eggs stripped from a ripening female. Fortunately, the spawning act is not necessarily inhibited by captive conditions in hatchery spawning ponds.

The first comprehensive account of sole egg and larval development was published in 1905 by Fabre–Domergue and Biétrix. The fertile egg is buoyant (1.5 mm diameter) and has a very narrow perivitelline space, like the plaice. The yolk contains characteristic clusters of so-called 'oil globules', giving a yellow-green tinge to the eggs. Incubation time varies with temperature, lasting 10–11 days at 11–12°C (Cunningham 1890), and 5–7 days at 13–14°C (Butler 1895). The newly-hatched buoyant larva (3–4 mm long) has an inflated yolk-sac, negligible eye pigment and an occluded pharynx. The eyes are fully pigmented, and the jaw articulation complete, 5–7 days after hatching at a water temperature of 11–12°C. By this time tank larvae, without exception, are to be found close to the bottom, grubbing around for food. Though of small size, the sole larva is relatively large-mouthed and capable of taking *Artemia* nauplii without difficulty. During subsequent symmetrical stages of development, the early benthic habit gives way to a very active pelagic life. The first signs of approaching metamorphosis occur three weeks after hatching, at a length of 9.5 mm, in temperature conditions slowly rising from 12°C to 14°C. The left eye migrates to the right side, now the heavily-pigmented dorsal surface, and structural metamorphosis is completed within 14 days, at a mean length of 10–11 mm.

Information regarding the requirements of sole larvae in the laboratory is of infrequent occurrence in the literature—a circumstance perhaps reflecting the difficulty of securing a successful artificial fertilization. Attempts to rear sole fry on a small scale have been recorded by Fabre-Domergue and Biétrix (1905), Dannevig (1948) and Flüchter (1965). Flüchter's Helgoland experiments, in small glass aquaria, lasted for a period of 34 days from egg liberation to metamorphosis at water temperatures between 15°C and 17°C. Using *Artemia* nauplii as larval food, he produced a count of 152 metamorphosed fish from 180 pond-spa eggs, giving a survival rate of 84 per cent at a survivor ity of 1 520 fish per m^2 of tank bottom. Flüchter's wor contemporaneous with similar studies on the Isle of which are described below.

Experiment H, 1965: Preliminary work in open circulation conditions

Aim To examine the possibilities of rearing the so metamorphosis in laboratory conditions.

On 8 May, a population of freshly-trawled ripe soles transferred to a $3.65 \times 3.65 \times 1.22$ m fibre-glass spaw pond in the hatchery, irrigated by unfiltered seawate 12°C. Fertile eggs appeared at the pond surface two after transfer, and egg production continued throughou months of May and June.

First-liberated eggs were of very poor quality ind indicating acute physiological stress among the spaw stock following capture. By the end of May, however, survival had improved to the point when rearing exp ments could be undertaken, with an expectation of rew ing results.

Procedure Three standard tanks ($60 \times 30 \times 30$ cm) cont ing antibiotic-treated seawater to a depth of 20 cm w stocked with egg or larval material at the rates quote Table 8. Tanks were sited in a temperature-controlled hat ery space and during the larval phase were irrigated a slow rate of flow (5 per cent tank volume/hour) with filte seawater. Overhead fluorescent lighting produced an tensity of 500 mc at the water surface, as measured wit photometer corrected by filter to the wavelength response the human eye. Food was withheld until the hatched lar possessed heavily-pigmented eyes, functional jaw articu tion and well-formed pectoral fins. Thereafter, *Arte* nauplii were fed daily to excess. The tanks were cleaned a the dead counted at frequent intervals. After preservati metamorphosed survivors were examined for size, dor pigment and bitten fins.

Results Survival and other data are presented in Tabl and Figure 16. Development from egg liberation to me morphosis is faster for a sole population (approximat 650 day/degrees) than for the plaice (approximately 8 day/degrees), assuming good tolerance to prevailing wa temperatures. Survivals exceeding 50 per cent and approa ing 80 per cent of original eggs, in the arbitrary tank con tions of Experiment H, suggest that the larval sole is m adaptable to the hatchery environment than the larval plai and is therefore a promising subject for fish-farming studi High population densities exceeding 4 000 metamorpho fish per m^2 are tolerated without the marked increase mortality rate so often a characteristic of plaice performa in crowded conditions.

Larval loss in tank 15 between day 23 and 27 after hat ing was preceded by general hyperactivity and distress; cause remains unknown but the symptoms and short du tion suggested an attack of epidemic proportions by pathogen, either in the hatchery seawater circulation introduced with the food. The high survivals of sole lar

nks 13 and 14 can be explained partly in behavioural s; they are not aggressive towards one another (note the incidence of bitten fish in Table 8) and incur little risk of ical damage in the narrow confines of small rearing s on account of their controlled 'hovering' action during l capture, which is in marked contrast to the high-speed cs of plaice larvae.

LE 8 *Survival and other data from a preliminary sole-rearing investigation; Experiment H, 1965*

	Rearing tank		
	13	14	15
	Stocking rate		
	1 000 newly-hatched larvae	1 000 gastrulae	1 000 blastulae
hed eggs	—	977	947
blished feeders	869	824	900
ivors			
) metamorphosed	799	764	581
) pelagic	2	2	—
ation × temperature day/degrees)	649.9	680.6	633.0
ivor density amorphosed fish per m²)	4 438	4 244	3 228
ivor length (mm)			
inimum	9.0	9.0	9.0
aximum	15.5	15.5	17.0
ean	12.3	12.6	12.8
andard deviation±	1.1	1.1	1.4
n percentage pigment survivor	93.5	94.3	80.1
entage frequency of nally-pigmented fish	92.6	91.9	65.2
entage bitten fish	0.9	0.5	3.1

s mentioned earlier in this text, dorsal pigment peculiari- occur commonly among metamorphosed plaice popula- s reared in the laboratory. The hatchery environment affect sole larvae in a similar, though generally less sev- manner. At the end of the sole rearing experiment each served survivor was measured, and an assessment made eye of its percentage dorsal pigment cover. In Table 8 the n values for dorsal pigment and the frequency of nor- ly-pigmented fish in tanks 13 and 14, both of which en- ed an uneventful larval phase, are greater than might be ected from eggs produced by a recently-caught plaice k. The lower values for tank 15 do not necessarily relate he unfavourable environment between day 23 and 27; egg stock probably came from a different spawner from supplying tanks 13 and 14. With a small spawning popu- on, egg production on a particular day will be dominated a particular fish; this is especially likely in the case of a cies with a protracted, rather than an explosive, spawning on.

he basic information on pigmentation was then analysed erms of (a) the mean percentage dorsal pigment and (b) proportional frequency of normally-pigmented fish per ivor length-group. Values for tanks 13–14 and tank 15 plotted separately on log/log coordinates in Figure 17, r some amalgamation of data at extremes of size range, n the numbers of examined fish were small. The regres- s are fitted by eye. The Y values for tanks 13–14 show e variance about the regressions on the basis of either n percentage pigment per survivor or the frequency of normally-pigmented fish. The data for tank 15, in respect of normally-pigmented fish, do not conform to a straight line regression; however, on balance, it seems from the evidence available at the moment that a similar correlation exists between pigment distribution and survivor size as has already been defined for the plaice. The tendency among a hatchery-reared sole population is towards better pigmentation with increasing survivor size (or faster rate of development).

The large-scale production of metamorphosed soles

The first mass-production exercises with soles was delayed until the spring of 1967, due to faulty larval food supply during 1966. By this time the resident population of adult soles was well acclimatized to captive conditions and had been fed on shucked 'queens' (*Chlamys opercularis*) since the summer of 1966, at a rate varying between 5 and 10 per cent of total body weight per day. In addition, the spawning pond temperature was never allowed to fall below 8°C during the winter months. Under these conditions, spawning started on 12 March 1967 at 10°C, one month earlier than expected. All floating eggs were removed from the pond each morning during the spawning season.

Procedure Two separate batches of eggs, comprising the amalgamated daily collections for 19–20 March and 2–6 April, were incubated at an ambient temperature of 10.0–10.5°C with gentle aeration in 120 × 60 × 30 cm black, polythene tanks containing static, filtered seawater treated with a single dose of mixed antibiotics (50 i.u. benzylpenicillin and 0.05 mg streptomycin sulphate per ml). At this temperature, incubation lasts 7–8 days; on the fifth day, weighed egg batches (now in the gastrula phase of development) were transferred to two 4.8 × 1.2 × 1.2 m sectional fibre-glass tanks (Plate 4) filled to a depth of 106 cm with static, filtered seawater (no antibiotics). The weight/number relationship was determined from spare egg samples. The tanks were sited in a hatchery space with dim natural lighting from roof windows; no attempt was made to control water temperature.

Artemia nauplii were offered as soon as larval samples indicated one or more individuals ready to feed. Subsequent naupliar rations were adjusted each day to avoid a build-up of uneaten food, each ration being counted electronically (Mitson 1963). Despite a slow accumulation of faecal material in each tank, cleaning operations were deliberately restricted to once per month during the course of the exercise. For the first three weeks of larval life the tanks were kept static to conserve naupliar food; thereafter they were irrigated with a slow flow (120 litres/hour) of filtered seawater for 12/24 hours at night only. Oxygen demands were met by gentle aeration, once feeding was well established. In both tanks metamorphosis started during the fifth week of life and was complete a month later. Daily rations of *Artemia* nauplii were then gradually replaced by another living food, the enchytraeid oligochaete *Lumbricillus rivalis*, which has proved to be an excellent diet for young flatfish immediately after metamorphosis. It occurs abundantly in decaying seaweed at the spring tide mark; techniques for cropping and cultivating this worm formed an important part of the research programme at Port Erin.

Results A resumé of production data is given in Table 9. It indicates that the rearing of young soles in numbers sufficient to support a large-scale cultivation enterprise is feasible, without the installation of complicated and expensive control equipment, or the employment of a large labour force.

The stability of dorsal pigmentation in the sole, already remarked upon during the discussion of experimental results for 1965, was confirmed by the low incidence of pigment deficiency at survivor densities exceeding 5 000 per m^2 of tank bottom. Food and temperature data are presented in Table 10. The comparatively poor food conversion rate (of the order 13:1 wet weight) is not explained completely by larval mortality after first-feeding, and probably reflects inefficient utilization of ingested food by sole larvae in conditions of plentiful supply, and hyperactivity induced by a high degree of crowding.

TABLE 9 *Mass-production of metamorphosed soles, 1967*

	Stock survival	
	Tank 5	Tank 6
Duration (days)	95	110
Egg stocking rate	77 400	57 800
Metamorphosed survivors	32 650 (42%)	25 710 (44.4%)
Survivor density (number per m^2)	5 487	4 314
Survivor size (mm)		
Mean	16.9	20.6
Range	12–26	14–30
Percentage of normally-pigmented fish	99	95

TABLE 10 *Mass-production of metamorphosed soles, 1967; feeding schedules and conversion rates on a diet of* Artemia *nau*

Food and temperature

Tank 5

Week	Mean temp. (°C)	Mean daily food	
		Artemia nauplii $\times 10^6$	*Lumbricillus* (g)
1	10.0	—	—
2	10.5	—	—
3	11.3	1.9	—
4	11.6	2.7	—
5	11.7	7.3	—
6	10.9	11.0	—
7	10.5	9.7	—
8	10.8	6.4	—
9	12.0	12.9	—
10	12.7	12.0	41.7
11	14.0	6.7	57.1
12	13.6	6.0	92.8
13	13.6	6.0	100.0
14	13.8	5.6	85.7

Tank 6

Week	Mean temp. (°C)	Mean daily food	
		Artemia nauplii $\times 10^6$	*Lumbricillus* (g)
1	10.1	—	—
2	10.3	0.1	—
3	11.0	1.0	—
4	12.0	2.0	—
5	10.8	4.0	—
6	11.6	5.5	—
7	11.3	7.8	—
8	11.1	8.6	—
9	10.5	7.3	—
10	10.9	9.1	—
11	11.8	12.8	—
12	12.4	15.7	45.7
13	13.8	8.3	53.6
14	13.7	6.1	92.8
15	13.7	6.0	100.0
16.	13.8	2.7	43.0

Food conversion rate

Tank 5

	Number $\times 10^6$	Wet weight (g)
Total *Artemia* nauplii	597.5	9 914.5
Total *Lumbricillus*		2 625.0
Total food supplied		12 539.5
Fish production		951.8
Food conversion rate		13.1:1

Tank 6

	Number $\times 10^6$	Wet weight (g)
Total *Artemia* nauplii	686.2	11 386.3
Total *Lumbricillus*		2 365.0
Total food supplied		13 751.3
Fish production		1 220.7
Food conversion rate		12.6:1

rends in Marine ish Culture

demand for high-grade protein must increase as the an population expands. A corresponding intensification he total world fishing effort would seem to be inevitable , since the productivity of the open oceans is finite and nges little over the short term, our national fishing indus- can expect to operate in an increasingly competitive cli- te.

A modern British deep-sea trawler costs at least £500 000 70 price) to build and is expensive to operate. During its ited life it travels vast distances to exploit a fluctuating ural resource commonly owned and often imperfectly nserved. This makes doubtful economic sense, and it is y to see why subsidy arrangements and imports of sea n or fish products are necessary to maintain our national quirements. Whilst manned ships are used to reap the s, fish production remains a labour-intensive industry, d this puts the more affluent nations in an increasingly or competitive position.

In such conditions international disputes over fishing hts become commonplace, and are not always solved by ulti-lateral agreement. A nation may find itself barred m customary fishing grounds; a long-established fishery ay fail through natural or other causes; and local shortages cur wherever stocks are fished irrationally. Uncertainty out the magnitude of future fish supplies will continue as ng as we place total reliance on the natural resource. oner or later, sea-fish farming must be given serious con- deration as a supplementary method of fish production.

e culture of sea fish in Japan

ne might expect the precocious development of economic arine fish farming to occur in those countries with a tradi- nally high *per capita* consumption of fish. This is the case Japan, where the valuable pelagic 'yellow-tail' (*Seriola inqueradiata*) has been fattened in captivity by individual hermen since the early years of this century. What was iginally a subsistence-type farming has now emerged as a bstantial national industry, largely as a result of govern- ent funding and organization since 1953. A stranger to pan cannot fail to be impressed by the present scale of pital investment in this new enterprise, even though the tput of cultivated fish and shellfish comprises a mere 2 per nt of the total annual catch from coastal, offshore and stant-water fisheries.

Ninety per cent of all yellow-tail eaten in Japan come from cultivated source. The species is farmed exclusively in the to Inland Sea (southern Honshu) where summer water mperatures reach a maximum of 28–29°C, falling to 8°C in winter. The industry is based on the intensive ttening of wild fry on 'gathered' food, through one sum- er season of rapid growth. As yet, no technique is available r propagating yellow-tail fry in shore hatcheries, though renuous efforts are being made to overcome technical fficulties.

Captive fish are held in a variety of enclosures ranging from suspended netting 'tanks' supported from floating rafts (Plate 5) to extensive shoreline impoundments of wire or synthetic mesh curtains, arranged on piles driven through sand to bed rock (Plate 6). A 300-acre bay at Matsumigaura in the north-west corner of Lake Hamana has been enclosed by a 300 m netting barrier placed across the entrance (Plate 7). A local fishermen's cooperative manages this marine ranch, apparently at a profit, though methods are very empirical indeed.

The main markets for yellow-tail are concentrated in the production area. In 1964, 700 firms were engaged in active cultivation, raising a total of 18½ million wild fry to a total market weight of between 15 000 and 20 000 metric tons (Rep. Japan Fish. Assoc. 1966). Captive fish are fattened on locally-caught trash fish species (sandeels, anchovy) and on waste mollusc tissue, at a conversion rate of approximately 8 (food):1 (edible fish).

Other examples of marine fish husbandry

The Japanese system for yellow-tail production is an 'open' technique in the sense that operators rely on seed stock collected from the sea. It therefore can be characterized as a redistribution of naturally-propagated populations, a form of human intervention having its parallels in other parts of the world. As early as 1892 Dr. C. G. J. Petersen, then Director of the Danish Marine Biological Station, transplanted young plaice from overpopulated nursery grounds in Danish coastal waters to richer feeding areas in Limfjord. His exploratory work was followed by regular annual transplantations on a large scale from 1908 onwards (Johansen 1928a, b). According to Blegvad (1951), the greatly increased growth rate of transplants made the whole scheme economically viable—an optimistic judgement in view of the subsequent decline in transplantation activities.

In some cases, completely new fish species have been introduced into areas where food resources were being inefficiently exploited by existing stocks. The transplantation of shad from the east to west coasts of the North American continent in 1873 was a particularly successful example of this technique. The striped bass or rockfish, *Roccus saxatilis* (Walbaum), was similarly transferred from east to west with equally gratifying results. Salmon and trout transplants from Britain in 1868 eventually gave rise to new and thriving fisheries in New Zealand, and, more recently, the Aral Sea has been colonized artificially with Baltic herring (Karpevich 1960, Bibov 1960). Lake Quarun, Egypt, now supports a commercial sole fishery as a result of transplantations started in 1938 (Salah El-Din El-Zarka 1963).

The redistribution of natural stocks by transplantation techniques, so as to exploit more fully the food available to fish, is theoretically desirable. In practice, however, there are distinct economic disadvantages; demersal transplants are easily damaged beyond recovery during capture in the trawl, and there is no guarantee that a migratory species such as the plaice will not be caught later, as a marketable fish, by vessels of other non-participating nations. At present, large-scale transplantation procedures are hardly likely to command national interest.

Another form of fish husbandry, the mass liberation of hatchery-reared offspring to augment natural recruitment, has already been mentioned. Despite a discouraging prognosis from former experience in the western world, the

Japanese are presently devoting a proportion of their available research effort to techniques for propagating non-migratory species of sea fish in hatcheries, with the declared aim of restocking the Seto Inland Sea, which is fast becoming depleted of its more commercially valuable fish. Any world-wide revival of interest in artificial recruitment must be preceded by systematic field experiments to establish beyond doubt that this form of human intervention can really improve the local yield of food fish. To this end Gunnar Dannevig (1963) released 100–150 million yolk-sac cod larvae into the heavily-exploited Oslofjord for a number of alternate years after 1950. His analysis of fishing records suggested a positive correlation between the abundance of 0-group cod in the littoral region and hatchery liberations. None of the years in which larvae were released yielded substantially smaller catches than the best of the intervening years.

It is known that near-water plaice stocks recuperate rapidly during periods of relaxed fishing effort (Borley 1923, Margetts and Holt 1948), suggesting that our coastal waters could support more fish than they do in their present heavily-fished state. However, it is a remarkable fact that although wartime stocks of plaice in the North Sea multiplied to several times their pre-war abundance, the recruitment of young brood from the heavy spawnings of 1945 and 1946 was no higher than average. Beverton (1960) has described the early environment of the young pelagic plaice larva as a kind of turnstile, permitting only a certain number to pass through, irrespective of the length of the queue behind. Not all the limiting factors in this environmental turnstile are known, but food and predator abundance are undoubtedly involved. The absence of both these limitations in the hatchery environment probably accounts for the gap between high experimental survivals and low estimates for the sea.

The first recorded attempt to cultivate marketable marine fish in Britain was made by Dr Fabius Gross of Edinburgh University (1947a, b; 1950a, b) and his co-workers (Orr 1947, Marshall 1947, Raymont 1947, 1950, Nutman 1950, Gauld 1950). They raised the basic fertility of two Scottish sea-lochs, Loch Craiglin and Kyle Scotnish, with monthly applications of common agricultural fertilizers (superphosphate, sodium nitrate and ammonium sulphate) in quantities sufficient to raise the phosphate concentration to 30 mg P_2O_5/m^3 and the nitrate to 100 mg N/m^3 of loch seawater. This treatment produced impressive increases in the seasonal abundance of plankton and benthos, followed by an equally remarkable acceleration in the growth rate of transplanted and resident fish stocks. Buljan (1961), using a refined fertilizer application technique in Adriatic bays, similarly improved both the survival rate and growth rate of wild shellfish populations.

Because of the migratory habits of fish and an inability to provide himself with enough young transplants, Gross failed to elucidate one of his main research objectives—the relationship between the quantity of fertilizer added and the subsequent yield of fish. There is a good case for repeating this classical work in a suitably screened sea-loch, now that a large-scale plaice-rearing technique has been developed.

larine cultivation: esearch nd development

ıtegy—a scientific view

long-term strategic aim of any commercial enterprise is ɔroduce a commodity for general consumption at a ıpetitive price. In the case of marine farming, thorough sideration of economic imponderables must await the wers to a range of fundamental bio-engineering problems. ɔur present state of knowledge a realistic research and elopment strategy might take the following form:

"To develop basic techniques for cultivating marine food organisms in a variety of exploitable environments, both natural and artificial, within the British Isles."

A basic technique is seen as one recommending itself to ɔlic or private enterprise by virtue of its economic possi- ties; economic refinement is a likely outcome of indus- ıl practice. The term 'marine food organisms' might lude fish, crustacea and molluscs acceptable to the British blic, or suitable for export. It should not be restricted to ligenous fauna if environments particularly favourable to ɔtic species can be provided, always supposing that ade- ate precautions against the introduction of new diseases e adopted. Species commanding a good market price and ɔwing *a priori* evidence of response to domestication chniques would seem to be the best material for priority ɔrk.

Broadly speaking, the natural environments worthy of nsideration are sheltered sea-lochs such as occur in Scot- nd and Northern Ireland; the artificial habitats are, at the oment, restricted to the warm condenser outflow from ıclear and conventional power stations. Some of the larger astal generating units are discharging as much as 50 illion gallons of seawater per hour at 7–8°C above intake mperature. As our technological society expands, the eater demand for power is being met by a proliferation of nerating stations in coastal situations, partly to conserve e national resource of fresh water for domestic and special dustrial use. Waste low-grade heat energy is difficult to cover except in a biological manner—for instance, by en- ɔling farm stock to maintain growth during the winter, thus aching marketable size at an earlier age. The gain is then terms of annual output per unit of capital expenditure on rm installations, rather than increased efficiency of food ɔnversion.

As a location for marine farms, the west coast of Britain ems more favourable than the east, from the standpoint of xposure to wave action, turbidity of inshore water and inter temperatures. When the artificial environment offers ıe best chance of progress, then substantial effort should be ɔncentrated in that quarter, since a quick commercial suc- ess in controlled conditions would do much to underpin ɔnger-term projects designed to bring more complicated atural and semi-natural habitats into a state of cultivation.

Types of fish cultivation

The ideas set out in this discussion apply mainly to carnivorous marine fish and crustacea. Herbivorous fish such as the mullet command a limited market in Britain. A molluscan programme, particularly for the oyster, would differ in several important respects, since bivalves already have a long history of industrial cultivation and associated research. Before considering the tactics of a research and development programme, it is necessary to define the type of fish cultivation most likely to succeed in a particular environment:

1 intensive production on 'gathered', cultivated or artificial food in strictly controlled conditions; appropriate to the power station environment, or regulated pond systems;

2 low-density 'ranch' production on mainly natural food in lightly controlled conditions, with or without nutrient enrichment; appropriate to sea-lochs enclosed with specially constructed wire or synthetic-fibre fish screens;

3 semi-intensive production on natural, gathered and artificial food, in more controllable conditions than (2), with or without deliberate enrichment; appropriate to extensive intertidal areas cut off by solid barriers, estuarine enclosures and moored cages.

Whereas types 1 and 3 involve the transformation of 'waste' protein into a more consumable or marketable form, type 2 sets out to actually increase the amount of high-protein food locally available to fish or crustacea by the simple expedient of ensuring that nutrients are never limiting to the productivity of an enclosure. The type of cultivation best suited to a particular locality will depend not only on the topography, hydrography and fish food supply of the region, but also on the availability of capital, labour, power, engineering services, research facilities, etc.

Programme priorities

The precise nature of effective research and development will depend upon the type of cultivation involved. The relatively simple structure of power station cultivation gives immediate freedom to concentrate on those factors likely to limit commercial production, with the prospect that a high degree of environmental control and predictability will eventually be achieved. A tactically different type of programme is called for in fish 'ranching', where even limited control over production would presuppose a quite extensive knowledge of the more important ecological interrelationships. This does not imply that movement towards cultivation in the natural environment must be preceded by elaborate ecological investigation, but merely that due regard must be given to ecology in any development programme.

Even though the special characteristics of an exploitable environment will determine the likely mode of cultivation, which in turn will require its own special tactical approach, there still remains a general framework of priorities applicable to all aquacultural projects. They concern:

(a) engineering feasibility, site planning and equipment design;

(b) biotic and physico-chemical factors affecting production.

No cultivation programme can start if fundamental engineering problems are insuperable; the subsequent speedy evolution of an efficient technique demands continued close liaison between biologists and engineers. The exact character of an engineering programme is usually seen in retrospect only, since it is essentially a process of adapting design, from data provided by biologists, to meet the often inflexible requirements of captive stock.

The main purpose of this dissertation is to consider where specifically biological effort might best be concentrated in order to achieve the stated strategic aim. The important areas of enquiry can be arranged in order of priority, based on the way in which they might operate to limit industrial production, supposing that no engineering impediment exists:

primary limiting factors:
(i) the provision of high quality 'seed' stock;
(ii) food supply for captive stock;

secondary limiting factors:
(iii) physico-chemical conditions;
(iv) stock/space relationships;
(v) disease;
(vi) predation;
(vii) competition.

The first five limiting factors are common to both natural and artificial environments; the influence of factors (vi) and (vii) will be minimal in the controlled environment. Answers to specific problems within some or all of the above research areas will, as the information accrues, influence equipment design and farm-site management procedures.

Research tactics: a general review

In generalized dissertations of this sort the furthest one can go in pursuit of biological research priorities is to give precedence to work on the primary factors limiting industrial production. The secondary limiting factors have a modulating effect on production under way and will vary in importance according to farm-site circumstances. For example, competitor and predator research would rank high in the research programme for screened sea-lochs, but would have low priority in power station cultivation. The following notes are intended as a brief resumé of the studies appropriate to the three modes of marine cultivation.

The provision of high-quality 'seed' stock The principal requirements in any form of animal husbandry are (a) young stock, and (b) food to nourish that stock. The cultivation of the yellow-tail in Japan is based on wild fry which occur offshore in easily-fishable concentrations. Where the natural abundance of fry can support industrial cultivation, the problem of stock supply might best be solved by research into catching gear and transport methods. Even so, farm production based on wild fry will be susceptible to long- and short-term fluctuations in the natural abundance of young, thus making it difficult to rationalize expenditure on production plant. There is always a tendency to cultivate the more valuable species, and high market value often reflects inadequate natural supply. In these circumstances the hatchery propagation of young stock commands high priority in research programmes; the developing role of the hatchery in oyster cultivation is a case in point.

Perhaps the most important long-term aspect of any ine cultivation programme is the establishment of sc genetical type-strains, particularly suited to specific environments, by enlightened selection procedures or c forms of genetical manipulation (Purdom 1970). The ar gies from animal husbandry are too obvious to dwell u Some controlled environments might be suitable for tinuous rather than seasonal cycles of production, but will only be possible when techniques for reproductory trol of spawners are more firmly established than at pres for a wide range of aquatic species.

Food supply: intensive cultivation The ideal artificial f for intensive (or semi-intensive) fish and prawn cultiva would:

(a) be cheap,
(b) be storable in the dry state,
(c) be acceptable to stock at all stages of developmer
(d) support maximum survival and permit fast growt
(e) have a high conversion ratio,
(f) induce good palatability in farm produce,
(g) act as a vehicle for prophylactic substances to com disease.

Compounded foods of this type are already available freshwater fish farmers in some parts of the world; comp able foods need to be developed for marine stock after d consideration of their nutritional requirements. Perhaps r all the criteria can be met; so far, flatfish larvae seem unal to utilize artificial foods and have only been reared succe fully on a live diet.

The problems of food supply, particularly for marine fis are best considered under three separate headings corr sponding to phases in their life-history:

1 larval,
2 transitional (the weaning period after metamorphosis
3 juvenile and beyond.

In the absence of a compounded food to satisfy stock at a stages of development, additional research effort could us fully be deployed on the experimental and large-scale pr duction of live food organisms for larval fish and prawn supplementary to the widely-used *Artemia* nauplius.

Direct weaning from a live to an inert diet at the newl metamorphosed stage accelerates the mortality and inhibi the growth of plaice and soles. Such problems can be ove come by offering larger live food organisms, intertidal an terrestrial oligochaetes for instance, until fish growth ha proceeded to the point where inert material becomes mo acceptable. Research into the culture of 'fodder' foods suc as mussels would be worth pursuing as a logical extension c molluscan cultivation programmes. If the post-larval growt of captive fish is dependent on gathered animal protein, as the case in Japan, it would be in the interests of farm proc ucers to know more about the distribution and abundanc of this waste raw material on a national scale, and how t exploit it cheaply and rationally.

Food supply: ranch-type cultivation Although 'gathered' c compounded foods might be used to supplement the diet c stock in low-density systems, a more realistic approac would be to view fish-ranching as an exercise in pastora

andry consistent with the basic or heightened product- of an area in conditions of limited availability of labour. ious work has demonstrated that the natural productiv- f Scottish sea-lochs, for example, can be raised by the cation of inorganic manures. Evidently nutrient con- ations exert a limiting effect on organic production time to time in these marine localities. For inorganic ures we need to know which type, in what quantity and to apply them so as to render nutrient concentrations limiting, and then, having disturbed the natural ecologi- alance, how to manage this enriched system so as to get best economic return in the form of edible fish (and lfish).

k/space relationships Reliable information on opti- n stocking rates for farmed fish at all stages of develop- t is vital to efficient management. It would also be an ious advantage to know the minimum size of tank or e permitting stock to grow at a maximum rate in other- e ideal conditions. There is good evidence that the layan strain of *Tilapia mossambica* responds to pond size vell as population density (Chen 1965); also, the Japanese d it expedient to vary the size of pond according to the e of the cultivated prawn *Penaeus japonicus*.

n nature, the 0-group stages of several European flatfish cies are generally found close inshore, separated from the ler year-groups which live in deeper water. Supposing that effect of such zonation is to minimize intraspecific preda- n and hierarchical influences, then it may be found expedi- to simulate year-group separation in farm practice. erarchical patterns exist in fish tanks; we need to know w they might be rendered less limiting to population owth and survival. In freshwater pond culture it is stand- d practice to increase productivity by stocking a variety of ecies, each of which exploits a different food source, whilst ll being compatible in other respects. Mixed-species farm- g is to the likely form of future sea-fish cultivation in some eas—if prefaced by appropriate research on interspecific mpatibility.

ysico-chemical conditions Water conditions, though ven the status of a secondary limiting factor to industrial oduction in this paper, could, of course, inhibit production together in extreme circumstances of O_2 depletion or vere industrial pollution etc., but the assumption is made at abiotic influences would not be a feature of a selected te. Hydrological engineers are unable to plan farm lay-out ficiently in the absence of data on species tolerance to impor- nt physical and chemical variables; sub-optimal levels, even hen tolerated, may adversely affect growth rate. Research rogrammes for particular farm localities would normally clude environmental studies of this sort, accompanied by e development of early-warning systems for monitoring e environment, wherever stock is at risk due to unstable hysico-chemical circumstances.

isease When animals are intensively farmed, special pre- autions have to be taken against the risk of epidemic di- ease. Our scanty knowledge of disease—its causes, symp- oms and control—among marine fish species reflects a istory of limited cultivation. Fish and shellfish pathology a study requiring full-time attention by those best qualified adapt existing medical and veterinary techniques to the quatic milieu, with an immediate emphasis on prophylaxis. t the moment, even freshwater fish culturists, with their ong history of commercial production, frequently suffer catastrophic stock losses through disease, which might have been avoided by proper prophylactic precautions. In the marine hatchery it is often feasible to treat outbreaks of parasitic infestation among spawning stock with empirical 'dips' in dilute formalin or a solution of quinine hydrochlor- ide, but this is hardly applicable to large-scale commercial production. The use of oral medicaments in food, hygiene procedures, immunological techniques, quarantine control and slaughter measures to counter severe outbreaks are but a few aspects of disease research deserving attention, in addition to the breeding of resistant strains.

Predation and competition Pest control should be the princi- pal aim of predator/competitor studies, though it is unlikely that natural or semi-natural farm environments could ever be kept free from pests without continuous intensive effort. A second line of defence against predators might be to lower the vulnerability of stock to predatory pressure by with- holding release into infested waters until individuals have reached a threshold size—a notion which awaits appropriate prey/predator size studies, accompanied by the development of 'nursery' techniques for the on-growing of young stock to threshold size in predator-free conditions.

Unwanted seaweeds respond strongly to the application of inorganic manures in sea-lochs (Gross 1947b, 1950b). Competitor control by mechanical or chemical means would seem a subject worthy of attention, if the maximum benefit to useful plant production is to be expected from fertilizer treatment.

Summary

1 A series of empirical plaice-rearing experiments began in closed circulation at the Lowestoft Fisheries Laboratory in 1957. During the course of five successive spawning seasons, laboratory output rose from 1 to 2 807 metamorphosed larvae at a maximum survivor density of 336 young fish per m^2 of tank bottom. Survivals seldom exceeded 5 per cent of original sea-spawned eggs.

2 In 1960 a second series of plaice-rearing experiments in open circulation, using pond-spawned eggs, started at the Marine Biological Station, Port Erin, Isle of Man. Over three successive seasons the maximum survival rates from the early egg stage through and beyond larval metamorphosis increased from 10.3 to 65.7 per cent at a maximum survivor density of 3 650 young fish per m^2 of tank bottom. This advance was due to technical improvements in:

(a) bacterial control, using antibiotics, during egg incubation;
(b) tank illumination during early larval feeding;
(c) the temperature regime during egg and larval development; and
(d) rearing-tank design.

3 Stocking densities above 2 000 viable plaice eggs per standard ($60 \times 30 \times 30$ cm) rearing tank cause undue mortality rates, due to overcrowding, in the late stages of larval life; the mortality rate within a larval plaice population of mixed size but equal age can be diminished by culling that stock into component size (or development rate) groups, even when the mean population density remains unchanged.

4 At a stocking rate of 1 000 eggs per standard tank, continuous irrigation with sea water is not essential to good survival and can be replaced by a gentle aeration system which, in any case, is beneficial during and after metamorphosis. Plaice larvae show a remarkable tolerance to their own metabolites; evidently the principal attribute of irrigation, in the context of plaice-rearing, is oxygen transport.

5 A plaice larva four or five days after hatching can consume between 10 and 20 *Artemia* nauplii per day, at 8–9°C, rising to about 200 *Artemia* per day at the onset of metamorphosis (12–13°C). The rate of daily increase in food consumption falls during metamorphosis, but rises sharply after its completion.

6 Preliminary experiments with pond-spawned eggs of the sole (*Solea solea* L.) have given survivals to metamorphosis greater than 70 per cent of original eggs. Sole larvae are less aggressive than plaice; even at high survivor densities, exceeding 4 000 metamorphosed fish per m^2 of tank bottom, the incidence of bitten fish was less than 1 per cent. Dorsal pigment peculiarities, though of comparatively rare occurrence, are related to survivor length distribution, as in the case of the plaice.

7 Both the plaice and the sole show an encouraging response to large-scale hatchery production techniques.

8 The nature of a research and development programme appropriate to marine fish farming in Britain is discussed.

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